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Differentiation of Tobacco Etch Virus Strains Affecting Pepper.

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DIFFERENTIATION OF TOBACCO ETCH VIRUS STRAINS AFFECTING PEPPER

A Dissertation
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The Department of Plant Pathology and Crop Physiology

by
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To

my brother,

Palitha Ariyaratne

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Tobacco etch virus (TEV) causes an important viral disease of pepper (*Capsicum* spp.) in the Western Hemisphere. In this study, 36 isolates of TEV from the United States, Mexico, the Caribbean, Central America, and South America were mechanically inoculated in the greenhouse to selected pepper lines reported to have resistance to one or more TEV isolates. Goals of the research were to evaluate reactions of the resistant pepper lines to the TEV isolates, and to determine if the TEV isolates could be grouped into pathotypes based on their reactions in pepper lines chosen as differentials during the course of the study. Additional goals were to determine if dsRNA analysis and light microscopic evaluations of nuclear inclusions could be used to group and distinguish TEV isolates.

Definite trends were evident with respect to virulence of isolates and resistance of pepper lines through the course of the study. Certain TEV isolates infected most of the resistant lines while others infected very few. There were isolates representing many gradations between the extremes. Reactions of specific pepper lines to specific TEV isolates sometimes varied in the different experiments of the study, possibly due to temperature and / or light intensity effects on resistance during the different times of the year in which experiments were carried out. Therefore it was not possible to distinguish clear virus pathotypes, nor to choose pepper line differentials to separate the isolates into pathotypes. However, using eight pepper lines : Yolo Wonder as susceptible control, VR2, Magda, Jaloro, VR4, Delray Bell, PI 159236, and PI 152225, most TEV isolates could be grouped into general categories of high and low virulence, and certain unusual

isolates could be well defined. Regaring variability in resistance among pepper lines, Agronomico 10C-5, Delray Bell, VR4, Jaloro, and PI 152225 were resistant to many TEV isolates tested, and appear to be good sources of resistance for use in breeding programs. Additional traits studied did not distinguish TEV isolates. All virus isolates reacted similarly in dsRNA analysis. Three of 10 virus isolates tested induced distinctive nuclear inclusion types, whereas the remaining seven isolates shared a single inclusion type.

INTRODUCTION

Peppers (*Capsicum* spp.) are grown and consumed worldwide. In Louisiana, three major types of peppers are produced: Tabasco (*C. frutescens* L.), bell, and cayenne (*C. annuum* L.). They are an important constituent of many foods for flavor, vitamin C, color and pungency, and therefore, of great value for the food industries.

The Economic and Statistics Service of the United States Department of Agriculture reports that the United States produced an annual average of 261,000 tons of green peppers for fresh market and processing from 1978 to 1980. These peppers were worth over $109 million yearly. The five major pepper producing states are Florida, California, Texas, New Jersey, and North Carolina. New Mexico, Arizona ,Texas, and California produce most of the hot chili peppers in the United States (Greenleaf, 1986).

Tobacco etch virus (TEV) is a member of the potato virus Y group. It is one of the most damaging viruses affecting peppers in the United States (Black et al., 1991). Early infection reduces fruit set, size and weight, whereas late season infection has little effect on fruit size and weight.

The most effective and inexpensive way to control TEV is through host resistance (Barrios et al., 1971). Breeders must ensure that they are incorporating resistance to viruses present in areas where the cultivars will be utilized. Availability of sources of resistance is necessary for breeders trying to produce cultivars which are both virus-resistant and horticulturally acceptable.

Distinct host reactions by individual pepper lines to different TEV isolates strongly suggest the occurrence of TEV pathotypes (Zitter, 1972). Nelson and Wheeler (1981) proposed a host differential series for potyvirus strain identification. The unknown potyvirus is inoculated into several selected hosts and symptoms are compared with those induced by known viruses on the same host. Characteristic symptoms on one or more hosts matching those of a known virus can be diagnostic for the unknown potyvirus.

One goal of this research was to evaluate the reaction of Capsicum lines and cultivars previously shown to be resistant to some TEV isolates. The second goal was to attempt to establish virus pathotypes based on pepper host differentials, if differentials could be identified.

An additional goal was to determine whether virus isolates could be grouped based on dsRNA analysis and viral-induced inclusion bodies.

LITERATURE REVIEW

Tobacco etch virus (TEV) was described by Fernow (1925) and Johnson (1930). Jimson weed (*Datura stramonium L.*) was the first reported natural host of TEV (Chester, 1937). TEV has a broad host range; over 120 species in 19 dicotyledonous families are susceptible (Holmes, 1946; Schmelzer, 1967; Edwardson, 1974b; Weinbaum and Milbrath, 1976). A leguminous weed, *Cassia obtusifolia* L. (sicklepod), is a natural host of the virus in both North and South America (Anderson, 1954; Debrot, 1976; Demski, 1979). Other natural hosts of TEV include *Cirsium vulgare* (Savi) Tenori, *Chenopodium album L.*, *Linaria canadensis* (L.) Dumort., *Physalis* spp. and *Solanum* spp. Diagnostic hosts of TEV include: *D. stramonium*, *Nicotiana tabacum* L., *C. annuum*, and *C. frutescens*.

Host symptoms. The severity of symptoms in cultivated plants depends greatly on the host species, cultivar and virus strain. Symptoms vary greatly in severity from mild mottle to severe mottling, leaf puckering, leaf distortion, shoe stringing, and extreme plant stunting. Tobacco etch virus also may cause severe fruit distortion, uneven fruit ripening and discoloration (Weinbaum and Milbrath, 1976). TEV symptoms in pepper include: mottling, mosaic, distortion of leaves and fruits, and stunting (Johnson, 1930; Zitter, 1971). In tobacco, the leaves are narrow and show mottling and necrotic etching (Stover, 1951; Gooding, 1970). Some isolates of TEV can induce local lesions, systemic necrosis and lethal wilt in Tabasco pepper. The wilting reaction is controlled by a single dominant gene not present in non-wilting peppers (Greenleaf, 1959; Nelson and Wheeler, 1981).

In the root tissues of infected plants, phloem and cambium necrosis and degeneration of cortex cells and plastids occurs (White and Horn, 1965). In *D. stramonium*, the leaves show mottling, distortion and vein banding, and capsular spines may be reduced or absent. *Cassia obtusifolia* shows systemic symptoms including necrosis, mottling, distortion, stunting and reduced seed production (Demski, 1979). Infected tomato plants are stunted and their leaves are mottled and distorted (Johnson, 1930).

Virus properties. Tobacco etch virus is an aphid-borne virus with flexuous filamentous particles composed of non-segmented, single-stranded RNA and protein. The protein and RNA composition is about 95% and 5%, respectively (Damirdagh and Shepherd, 1970). The genetic map of TEV is similar to other potyviruses. At the 5' terminus there is a covalently linked VPg. The main features of the TEV genome are: a 5' non-coding region of 144 nucleotides rich in A and U, a single large ORF of 9161 nucleotide which could code for a polyprotein of about 3000 amino acids and a 3' untranslated region of 190 bases terminating in a poly(A) tract (Dougherty and Hiebert, 1980; Matthews, 1991). The particle length of TEV is about 730 nm, and the diameter is about 12-13 nm (Damirdagh and Shepherd, 1970; Brandes and Wetter, 1959). In tobacco sap, the thermal inactivation point of TEV is about 10 min at 55 C (McKinney et al., 1965) with a dilution end-point of about 10^{-4}. Infectivity of TEV in the sap is maintained for 5-10 days at 20 C. The sedimentation coefficient of TEV is 154 S (Purcifull, 1966). Buoyant density of TEV is 1.33 g/cm in CsCl, and the extinction coefficient at 260 nm

is 2.4 (Damirdagh and Shepherd, 1970). It is readily transmissible by sap inoculation, (Matthews, 1991; Hollings and Brunt, 1981) but transmission through seeds has not been reported.

Serology. Tobacco etch virus is strongly immunogenic, and liquid precipitation tests with plant sap or purified virus have been used for detection and for studying serological relationships (Chester, 1937; Bartels, 1964; Purcifull, 1964). Good results are obtained in immunodiffusion tests with particles degraded into low molecular weight diffusible antigen by ethanolamine (Purcifull and Gooding, 1970), acetic acid (Purcifull, 1964), pyrollidine (Shepard et al., 1974) or sodium dodecyl sulphate (SDS) (Gooding and Bing, 1970; Purcifull and Batchelor, 1977). The virus has been detected by the enzyme-linked immunosorbent assay (ELISA), a more sensitive test (Clark and Adams, 1977). In ELISA tests, serological reactions linked with enzymatic reactions take place in wells of a special plastic microplate. When the enzyme substrate is added, a color develops and the intensity is proportional to both the degree of homology between virus and antigen and to virus concentration.

Intracellular inclusions. Two distinct types of inclusion bodies, cytoplasmic inclusion protein (CI) and nuclear inclusions (NI), occur in TEV-infected cells (Kassanis, 1939; Sheffield, 1941; Edwardson et al., 1968; Edwardson, 1974a). They are products of the viral genome and are induced consistently with different hosts. Viral-induced inclusions may consist of altered host constituents, aggregated virus particles or coat protein shells and virus-coded proteins other than coat protein. Cytoplasmic inclusions can be differentiated from the surrounding cytoplasm and organelles of the infected plant

cell by their reaction to several stains (Christie and Edwardson, 1986; 1977). Their detection can provide a rapid and relatively inexpensive method for diagnosis of viral infections. In many cases, viral inclusions have such a distinctive appearance that they may be used to identify a specific virus group (Christie and Edwardson, 1977).

Cytological studies of inclusions can be carried out with light and electron microscopy. Shepard and Shalla (1969), using ferritin-labelled antibodies, confirmed that inclusions and viral coat proteins were serologically unrelated.

Initially, the NI induced by TEV were thought to be thin crystals and appeared as rectangular plates (Kassanis, 1939; Sheffield, 1941). Subsequent electron microscopic investigations indicated that they are truncate four-sided pyramids (Bawden and Kassanis, 1941). The host cells with intracellular crystals do not appear as a group but are scattered throughout the tissue. Therefore, adequate sampling is important. A nucleus may contain one or two crystals (Matsui and Yamaguchi, 1964). These crystals are variable in size, and their numbers increase with the age of the infection (Sheffield, 1941).

Immunodiffusion tests have confirmed that purified NI protein is serologically unrelated to TEV particles, cytoplasmic inclusion protein, and healthy plant proteins (Knuhtsen et al., 1974).

When properly stained, virus induced inclusions are of sufficient size to be seen with the light microscope. Unstained inclusions are hyaline and difficult to distinguish from the surrounding cytoplasm. Stains make structural details of the inclusions visible and distinguishable from the infected host constituents and therefore enhance their detection. Suitable staining procedures have been fully described by Christie and

Edwardson (1977). The NI can be readily detected by light microscopy in stained epidermal strips from the infected leaf. The easiest way of detecting the intranuclear inclusions is by examination of epidermal strips taken from leaves showing pronounced chlorosis (Sheffield, 1941). Inclusions are seen more easily if plastids are first dissolved by immersing the strip in 5% Triton X-100 for 3-5 min. Two stains are available, O-G and Azure A. The O-G stain is a combination of Calcomine Orange and Luxol Brilliant Green and is used to stain protein (Christie and Edwardson, 1977). The Azure A stain is a combination of Azure A and 0.2 M dibasic sodium phosphate and is used to stain nucleic acids.

With severe TEV strains, nuclei are often found with numerous NI, whereas with mild TEV strains the nuclei usually contain few inclusions (Kassanis, 1939). The NI usually appear to be flat crystals, 6-8 um square when viewed from above, but are slightly curved plates in side view. Not all the NI formed by mild TEV are flat plates; they may be eight-sided bi-pyramids (Kassanis, 1939).

Transmission. TEV is mainly found in solanaceous plants and is transmitted in a nonpersistent manner by over 10 species of aphids, including *Myzus persicae* (Sulzer), *Macrosiphum euphorbiae* (Thomas), and *Aphis fabae* Scop. (Kassanis, 1941; Kennedy et al., 1962; Edwardson, 1974a). Virus acquisition and inoculation probes of 10 sec each are sufficient for transmission (Taylor and Robertson, 1974). The virus is retained for only 1-4 h by feeding aphids (Kassanis, 1941), but a probing individual aphid can transmit the virus to as many as five consecutive plants (Taylor and Robertson, 1974). Third and fourth

instars, as well as adults, can transmit and pre-access fasting increases transmission. An epidemic of TEV in South Florida during 1970-71 occurred after a massive increase in aphid populations, mainly *M. persicae* (Zitter, 1971).

Double-stranded RNA. Approximately 90% of plant viruses contain single-stranded RNA (Matthews, 1991). The presence of high molecular weight double-stranded ribonucleic acid (dsRNA) in plant extracts infected with RNA viruses is well established (Valverde et al., 1990). In an infected host, replication of the nucleic acid requires the formation of a double-stranded RNA (dsRNA). Thus, analysis of dsRNA from infected host tissues can be used as a tool for diagnosis of viral infections (Valverde et al., 1990). DsRNA is commonly separated using high-resolution polyacrylamide gel electrophoresis (Sambrook et al., 1989). Members within the same virus group have similar dsRNA profiles. However, this method sometimes can be used to distinguish different viruses, and strains of the same virus (Valverde et al., 1990). Nevertheless, it is not practical for routine diagnosis of viruses that yield low amount of dsRNA such as potyviruses and luteoviruses (Valverde et al., 1990).

Geographic distribution. Tobacco etch virus occurs widely in the United States, particularly in the southeast and in Arizona, Texas (Villalon, 1975; 1985), and California (Nagai and Smith, 1968; Villalon, 1985). It also occurs in Canada (Kemp, 1978; Lana and Peterson, 1980), Hawaii, Mexico, Puerto Rico (Perez et al., 1974), El Salvador (Granillo et al., 1974), India (Bidari and Reddy, 1983; 1986), Sudan (Mills, 1987), and Cyprus

(Nicosia, 1979). It causes major losses in peppers in different areas of the world and causes severe epidemics in bell pepper production in the United States and other countries (Green and Kim, 1991).

A survey in California, by Abdalla et al. (1991), reported 36, 99 and 88% incidence of TEV in 1984 from 486 random samples collected from Ventura, Tulare and Imperial counties respectively. Commercial bell pepper fields in southern Illinois were infected with TEV during the 1971 growing season and in each succeeding year (Weinbaum and Milbrath, 1976). In the central region of Georgia, TEV was reported in pimento pepper (Demski, 1979; Kuhn and Dempsey, 1964). It was the predominant pepper virus disease in northeastern Georgia in 1983 and 1984 (Benner et al.,1985). In this region, TEV was isolated from perennial *Solanum* spp. and *Physalis* spp. located in and near pepper fields, suggesting that these hosts may be a source of primary inoculum of TEV (Benner et al., 1985). In North Carolina, TEV incidence approaches 70% each season (Main and Gurtz, 1988). Tobacco etch virus was one of the viruses most commonly found infecting peppers in South Texas (Villalon, 1975) and Florida (Zitter, 1971, 1972, and 1973).

In a survey of viruses in pepper fields in Louisiana, TEV, potato virus Y (PVY) and cucumber mosaic virus (CMV) accounted for over 90% of the infected plants (Sciumbato, 1973; Whitam, 1974). Virus diseases commonly found in commercial peppers grown in south Louisiana in the 1950's were mainly caused by TEV and CMV (Horn and Sinclair, 1959; Sinclair et al., 1957).

Disease losses. Tobacco etch virus has been found to reduce both fruit number and average fruit weight per plant (Nutter et al., 1989). Early infection reduced yield 74% in 1986 and 73% in 1987, while late season infection reduced yield 5% and 7%, respectively (Nutter et al., 1989). In 1989, TEV was identified as the most important virus infecting bell peppers in northeastern Georgia (Kuhn et al., 1989). None of the fruit harvested from infected plants was of commercial quality.

Resistance. The most effective way to control TEV is through host resistance (Padgett et al. 1990). TEV resistance had originally been described in *C. annuum* SC 46252 by McKinney (1952). Resistance in SC 46252 was recessive and monogenic. Cook and Anderson (1959) and Nagai and Smith (1968) reported resistance in *C. annuum* PI 264281 to TEV. This line possesses a recessive gene for resistance to TEV very similar to that of SC 46252 and at the same locus (Cook, 1960). Weinbaum and Milbrath (1976) found immunity in PI 264281 to TEV. A pungent pepper, PI 152225 from Peru, is resistant to TEV but not immune (Greenleaf 1953, 1959; Sowell and Demski, 1979). Accessions PI 152225 and PI 159236 (*C. chinense*), which are highly resistant to potyviruses, are reported to contain recessive genes that are thought to be either allelic or located at two closely linked loci (Subramanya, 1982). Greenleaf (1956) found resistance to TEV in PI 152225 and SC 46252 to be inherited as a single recessive gene with one or more modifying genes. Resistance in PI 152225 and SC 46252 was expressed as a reduced rate of virus multiplication in plant tissues compared with a susceptible host.

The symbols: et^c and et^a were assigned to the respective resistance genes to denote their species origin (Greenleaf, 1956). Cook (1977) reported TEV resistance in cultivar Florida VR2. Accessions PI 264281, SC 46252 and VR2 were resistant to TEV-C (common strain), and their resistance allele was given the designation "et^a" (^{a} for *annuum*). Accessions PI 152225 and Tabasco G (Greenleaf Tabasco) were resistant to TEV-S, a more virulent strain, and their resistance allele was given the designation et^{c2} (^{c} for *chinense; et^{c1}* was used to designate the resistance allele of PI 159236). According to Greenleaf (1956), the et^{c2} allele was dominant to et^a.

Greenleaf (1986), described the resistance allele from the Brazilian cultivar Avelar and designated it "et^{av}". This allele conferred resistance to a greater number of TEV isolates than et^a and was believed to be dominant to et^a. Avelar also was tolerant to pepper mottle virus (PeMV) and tolerance was controlled by the same et^{av} allele. Zitter reported similar levels of resistance to TEV-C in PI 159236 (*C. chinense*) and Avelar. One line of Avelar, (PI 410407) was highly resistant to TEV, while two other lines (PI 342948 and PI 264281) were significantly less resistant (Sowell and Demski, 1977).

TEV resistance reported in pepper cultivars Florida VR-2, Delray Bell, Avelar, Agronomico 8, and Agronomico 10 appeared to be inherited as a recessive gene with a number of modifying genes that determined the intensity of resistance. The number of modifying genes present may be influenced by the TEV-susceptible parent, as well as by the resistant parent (Greenleaf, 1956).

Greenleaf Tabasco (GLT), derived from a cross between Tabasco (*C. frutescens*) and two *C. chinense* lines PI 152225 and PI 159236, carries monogenic resistance to TEV (Greenleaf, 1970). Cook (1977) reported TEV resistance in Delray Bell obtained from crosses between Avelar (lines 23-1, 71-23, 71-24, 242, 441, and 29) and Early California Wonder. Subramanya (1982) reported that resistance genes of Delray Bell and PI 159236 were allelic. Barrios et al. (1971) reported a dominant resistance gene to TEV in a *C. frutescens* cultivar LP-1, which remained symptomless when inoculated with TEV. Cook (1982, 1984a) reported TEV resistance in a nonpungent *C. annuum* cultivar (Florida-XVR- 3-25) obtained by crossing the hybrid from the cross of *C. chacoense* PI 260435 and Lincoln Bell with *C. annuum* cultivar VR2. Florida VR4, a TEV resistant cultivar, was derived from Delray Bell (Cook, 1984b; Cook et al., 1977). Florida VR2-34, a selection from Florida VR2, also is resistant to TEV (Cook 1977, 1984c; Cook et al., 1976).

Kuhn et al. (1987, 1989) evaluated pepper lines in the field and greenhouse for their reaction to TEV. A line with PI 264281 in its background, GA-C44-V22, exhibited high resistance to TEV, while three others, FL-XVR-3-25, Tambel-2 and Asgrow-XPH5021, were moderately resistant. Two lines, FL-XVR-3-25 and GA-C44-V22 exhibited high resistance under greenhouse conditions, but under field conditions 50-85% of the FL-XVR-3-25 and 15-25% of the GA-C44-V22 plants developed mild symptoms. Moderate resistance in lines Tambel-2 and Asgrow-XPH-5021 was characterized by mosaic and little or no stunting under both greenhouse and field conditions. Breeding lines from the University of Georgia (C44NV and C44CA) were highly resistant with only mild

mottling in 10% of plants in the field. Resistance to TEV has been reported in FLBG-1, a cross between Cubanelle and Agronomico 8 (Subramanya, 1982). Casca Dura (PI 342949) was reported to be resistant to TEV in the greenhouse, but it was not resistant in the field (Sowell and Demski, 1977). The TEV-resistant cultivar USAJI 5 was derived from a cross between PI 265281 and Ecuadorian selection ECUAJI (Cook, 1982, 1984d). Villalon (1981, 1983, 1986a, 1986b, 1986c, 1986d, 1987, 1988, 1991, 1992) used PI 342947, Agronomico 8, PI 264280, PI 264281, AC2207, or Avelar as sources of potyvirus resistance in the development of the cultivars Tam Mild Jalapeno-1, Tambel-1, Tambel-2, Hidalgo, Tam Mild Chile-2, Tam Rio Grande Gold-Sweet, Rio Grande Gold, Tam Veracruz, and Jaloro.

Lower disease incidence has been observed in TEV-resistant peppers (Benner et al., 1985; Kuhn et al., 1989). According to Padgett et al. (1990), final TEV disease incidence was 45% less and apparent infection rate was 50% less in resistant genotypes Tambel-2 and Asgrow-XPH-5021 compared to susceptible Yolo Wonder B. The consequence of the rate-reducing resistance in Tambel-2 and Asgrow-XPH-5021 was to increase fruit yield (24%), weight (14%) and number of fruits when compared to susceptible Yolo Wonder B.

Resistance to various isolates of TEV has been reported in lesser known *Capsicum* species such as *C. microcarpum* Cav., *C. pendulum*, Willd., *C. pubescens* R & P., and *C. baccatum* L. (Singh and Chenulu, 1980, 1985; Horvath, 1986a, 1986b).

Cultural control. The influx of winged aphids and the incidence of aphid-borne TEV was greatly reduced for aluminum-mulched plots of *Capsicum*, and yields were increased two to four fold (Black and Rolston, 1972; Black, 1980). Kemp (1978) reported that TEV incidence in *C. annuum* was reduced 45-75% with sawdust mulch, 12-63% with corncob mulch, 72% with straw mulch, and 53% with woodchip mulch.

Strains. There is evidence that different strains of TEV exist. Smith (1970) and later Makkouk and Gumpf (1974) proposed a strain classification scheme in which six pepper cultivars could differentiate five TEV strains.

Reactions of TEV isolates collected from California, Texas, and Sinaloa (Mexico) on Tam Mild Jalapeno 1, Tambel-1, Tambel- 2, Tam Mild Chile 1, and Tam Mild Chile 2 indicated that the Sinaloa isolate was the most severe on bell and chilli types, while the California isolate was most severe on Jalapeno types. The Texas isolate did not affect the resistant lines significantly (Villalon, 1985).

Smith (1970) tested California isolates of TEV against PI 342947, Agronomico 8 (PI 342946), Avelar (PI 342948), and Yolo Y and found five different reaction types among the isolates based on their ability to infect the four lines. Line PI 342947 showed the widest range of resistance. Zitter (1972) inoculated 13 pepper lines (PI 152225, PI 264281, SC 46252, 23-1-7, Yolo Y, 23-1-7 x Yolo Y, Agronomico 8, Avelar, Ambato Immune, PI342947, PI 159236, AC-2207 and PI 281367) reported to be resistant to TEV using Florida pepper field isolates. Common isolates of TEV were able to infect only Yolo Y. Three isolates designated as TEV-S were able to infect 12 of the 13 lines. The only exception was PI 152225. In southern Florida, the common isolate of TEV infected

only Early California Wonder, Yolo Y and Tabasco, but severe strains of TEV infected Early California Wonder, Yolo Y, Agronomico 8, Avelar, Florida breeding line 23-1-7, and Tabasco in varying degrees in the 1971-72 season (Zitter, 1973). Avelar expressed the greatest amount of tolerance. These severe strains were recovered from the east coast counties of Florida (Zitter, 1973). None of the commercial cultivars studied over 3 years exhibited resistance to two strains TEV-A and TEV-C (Zitter and Ozaki, 1973). Sowell and Demski (1977) tested various *Capsicum* lines in the greenhouse and field against two isolates of TEV. Agronomico 8, PI 152225, Avelar and AC2120 were highly resistant to both isolates. Nagai and Smith (1968) collected TEV isolates from pepper, tomato, and datura in California. TEV isolates reacted similarly on pepper lines inoculated. Agronomico 8, PI 264281, AC2120, PI 152225, and PI 159236 were resistant to all the TEV isolates tested.

MATERIALS AND METHODS

All experiments were conducted in a greenhouse during 1993, 1994 and 1995 at Louisiana State University in Baton Rouge. Greenhouse temperatures ranged from 21-35 C during the summer and 15 -29 C during the winter days. The study consisted of three phases.

Phase 1

Thirty-six isolates of TEV from different geographic locations that had been identified by serology and host range were used (Table 1). Stock cultures of isolates were maintained at 4 C in dehydrated host plant tissues in sealed containers with anhydrous $CaSO_4$. Selected virus isolates were activated by inoculation into 3-wk-old datura (*Datura stramonium*) planted in methyl bromide-fumigated soil in 10-cm clay pots. Thirteen pepper lines and cultivars (Table 2), previously reported to be resistant to one or more isolates of TEV, were selected to evaluate resistance to the 36 isolates. Seeds were planted in black plastic, 64 cavity seedling flats (Jiffy Products, Batavia, IL) using Jiffy Mix Plus planting medium (Jiffy Products) and maintained in a greenhouse (Fig 1).

Ten days after inoculation, datura leaves showing virus symptoms were used as a source of inoculum. The inoculum was prepared by grinding 1 g of leaf tissue in 5 ml of cold, 0.025 M potassium phosphate buffer, pH 7.2, with sterilized mortars and pestles. The cold inoculum was applied with pestles onto carborundum-dusted leaves of 3-wk-old pepper plants. Sixteen plants were inoculated for each TEV isolate-pepper line or cultivar combination. In separate flats, 16 uninoculated plants of each pepper were maintained as negative controls.

Table 1. Geographic origin, host, and source of tobacco etch virus isolates used in Phase 1

Isolate	Geographic origin	Host	Provider and year of collection	
BR-TAB	Louisiana	Tabasco	L. Black[a]	1992
CAJ2A#1, C5	Louisiana	Cayenne		1991
B1	Louisiana	pepper		1972
B3	Louisiana	pepper		1989
CAY-90-2	Louisiana	Cayenne		1990
CAP'-86	Louisiana	Cayenne		1986
C1	Louisiana	Cayenne		1972
C3	Louisiana	Cayenne		1990
TOM-1	Louisiana	tomato		1975
DR-92-5	Dominican Republic	pepper		1992
DR-92-6	Dominican Republic	Cayenne		1992
DR-92-7	Dominican Republic	Tabasco		1992
DR-93-13	Dominican Republic	Cayenne		1993
DR-93-19	Dominican Republic	Tabasco		1993
DR-93-23	Dominican Republic	Cayenne		1993
DR-93-28	Dominican Republic	Cayenne		1993
V-92-4	Venezuela	pepper		1992
NW-C-86-4	Colombia	Tabasco		1986
H-93-5	Honduras	Tabasco		1993
H-92-31	Honduras	Cayenne		1992
MEX-21	Mexico	pepper		1979
TX-M	Mexico	pepper		1993
NW-M-81-1	Mexico	pepper		1981
NW-M-82-2	Mexico	Tabasco		1984
NW-M-83-1	Mexico	pepper		1983
LMS-M	Mexico	pepper	R. Christie[b]	1993
LMTP-M	Mexico	pepper		1993
SEVERE	Florida	tobacco		1989
ATCC-PV-69	Florida	unknown		1989
FL-978	Florida	tobacco		1989
MTP-C	California	unknown		1992
GLT-F	California	unknown		1992
15-D, 401	California	unknown	J. Watterson[c]	1982
VIL	California	unknown	B. Villalon[d]	1990

[a]Louisiana State University,
[b]University of Florida,
[c]PetoSeed Company,
[d]Texas A & M University.

Table 2. Pepper lines and cultivars used in Phase 1

Peppers	Species	Seed source	Year
PI 152225	*C. chinense*	H. Hobbs[a]	1992
PI 159236			1992
TF-38-A[b]		L. Black[c]	1993
136 A A Cook	*C. annuum*		1992
Avelar			1975
Casca Dura			1992
Tabasco Type Mex'88[d]	*C. frutescens*		1993
Greenleaf Tabasco		McIlhenny[e]	1992
Yolo Wonder	*C. annuum*	Peto Seed[f]	1992
Yolo Y			1992
VR2			1992
Agronomico 10C-5			1992
Delray Bell		A. A. Cook[g]	1992

[a]Louisiana State University.
[b]Selection of Asian Vegetable Research and Development Center line COO943.
[c]Louisiana State University.
[d]A selection of a Mexican Tabasco-type line.
[e]McIlhenny Company.
[f]PetoSeed Company.
[g]University of Florida.

Fig 1. Flats containing pepper lines used in the tobacco etch virus screening studies.

Isolates that induced similar reactions in the 13 peppers in Phase 1 were grouped. Representative isolates for each group, as well as severe, mild, or unusual isolates representing different geographical origins were selected to screen 33 pepper lines and cultivars in Phase 2. Both Phase 1 and 2 were conducted once.

Symptom evaluation. Symptoms were evaluated 3 wk after inoculation. Disease severity was scored using the following designations: NS=no symptoms, MM=mild mosaic, M=mosaic, SM=severe mosaic, and SMD=severe mosaic and leaf distortion (Fig. 2).

Enzyme-linked immunosorbent assay (ELISA). Presence of TEV in selected inoculated pepper lines with mild or no symptoms was tested for by ELISA (direct double antibody sandwich method) using commercial ELISA kits (Agdia 1000, AGDIA Inc., Elkhart, IN). For each isolate-pepper combination, 16 leaves were collected (one per plant). Samples consisted of the youngest fully-expanded leaves. Leaves were collected 3 wk after inoculation. In addition, leaves of uninoculated healthy plants and leaves of a susceptible cultivar inoculated with the same TEV isolate were collected as negative and positive controls, respectively. Leaves from four different plants were combined to obtain one 0.15 g sample. Therefore, four 0.15 g replicate samples from each isolate-pepper combination were used for ELISA testing. Plant sap was extracted from each 0.15 g sample in 1.5 ml extraction buffer =20.0 g of polyvinylpyrrolidone, MW 24 - 40,000, 1.3 g of sodium sulphite, 20.0 g Tween-20, dissolved in 1000 ml of 1 X phosphate buffered saline Tween (PBST), using a leafroller tissue grinder. One hundred microliters of extracted sap was placed in each well of the ELISA plate.

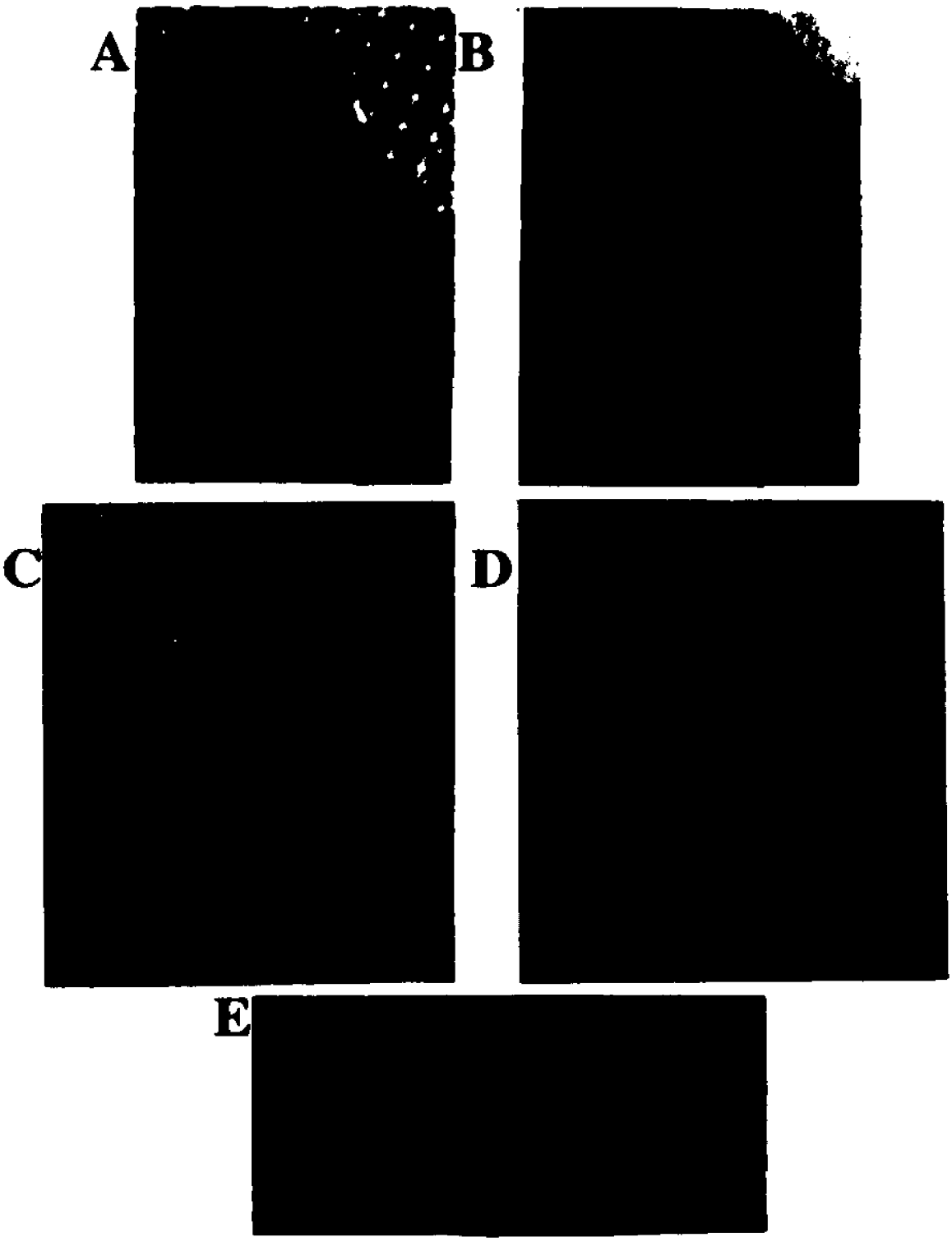


Fig. 2. Pepper leaves showing different symptom reactions after inoculation with tobacco etch virus. (A) no symptoms, (B) mild mosaic, (C) mosaic, (D) severe mosaic, (E) severe mosaic and leaf distortion.

ELISA plates were read with a Bio-Rad ELISA reader (model 2550) using a 405 nm filter. The threshold value used for determining a positive reaction was 2X the average absorbance value of the uninoculated control. The absorbance data was used to determine reactions of pepper lines to virus isolates. The level of ELISA absorbance is believed to reflect virus concentration. Comparison of ELISA absorbance was possible because of the standardization of tissue to buffer ratio, reagents, sample volume in wells, and incubation times.

Phase 2

Thirty-three pepper lines (Table 3) reported to be resistant to some potyvirus isolates were evaluated for their reactions to 10 isolates of TEV selected from Phase 1. Selected isolates were TEV-401, TEV-CAY-90, TEV-MEX-21, TEV-C1, TEV-TX-M, TEV-VIL, TEV-DR93-28, TEV-LMS-M, TEV-H93-5, and TEV-V92-4. Isolates were selected because they represented different levels of virulence, different geographic origins, or because they were unusual isolates. Sixteen pepper plants from each line or cultivar were mechanically-inoculated with each isolate of TEV as described in Phase 1.

Symptom evaluation and ELISA. Test plants were evaluated 3 wk after inoculation for symptom development, as described previously. As in Phase 1, selected symptomless, inoculated pepper lines were tested with ELISA.

Table 3. Pepper lines and cultivars inoculated in Phase 2 and seed sources

Pepper lines	Seed source	Year of collection
Veracruz Jalapeno	B. Villalon[a]	1993
Jaloro		1993
Rio Grande Gold		1993
Tambel- 2		1993
Tam Mild Jalapeno 1		1993
Hidalgo		1971
Tam Veracruz	PetoSeed Company	1993
ELS-2-1		1993
TSCH-2		1993
FLBG-1		1993
S-20-1		1993
King Arthur		1992
Yolo Wonder		1992
SC-46252		1992
VR4		1992
Magda	Rogers NK Seed Company	1993
Bomby		1993
Elisa		1993
Marquis		1993
Reinger		1993
Casca Grossa	PGRCU[b]	1993
Puerto Rico Wonder		1993
Agronomico 10C-5		1993
Agronomico 10G24-7		1993
Agronomico 8		1993
Avelar		1993
PI 264281		1993
Casca Dura		1993
Casca Dura Ikeda	AVRDC[c]	1993
CO-1664		1993
I-20 (AMA-12)		1993
92LB 444Q	L. Black[d]	1992
LP-1		1992

[a]Texas A & M University.
[b]Plant Genetic Resources Conservation Unit, GA.
[c]Asian Vegetable Research and Development Center.
[d]Louisiana State University.

Phase 3

Eleven pepper lines that showed potential as sources of resistance to TEV isolates were selected from Phases 1 and 2 (Table 4). Thirteen pepper lines (Tabasco and Yolo Wonder were used as susceptible controls) were selected and inoculated 3 wk after planting with the 10 TEV isolates used in Phase 2.

Symptom evaluation and ELISA. Test plants were evaluated for symptom expression 3 wk after inoculation. Leaves were collected from 16 individual test plants for each isolate-pepper line combination except for Tabasco. Leaves were pooled in groups of four, and a 0.15 g representative sample was obtained per isolate-pepper line combination. Serological tests using ELISA were performed as in Phase 1. Negative controls were included in the same ELISA plate for each pepper line. A separate ELISA plate was used for each of the 10 virus isolates, with 12 pepper lines x 4 wells = 48 wells used for infected samples and 12 peppers x 4 wells= 48 wells used for uninoculated control plants of the same pepper lines. In order to confirm the reactions of the 12 pepper lines to the 10 TEV isolates, Phase 3 was repeated.

Statistical analysis. A completely randomized design was used to compare the ELISA absorbance of 12 pepper lines inoculated with each of 10 isolates. Tukey's Studentized Range (HSD) Test was performed for each isolate, using the mean ELISA absorbance of 12 inoculated pepper lines. A 2 x 12 factorial analysis was performed on experiment 1 and 2 separately and on 1 and 2 combined. The SAS program was used for the analysis (SAS, 1995.).

Table 4. Pepper lines and cultivars and seed source used in Phase 3

Pepper lines	Species	Seed source	Year of collection
PI 152225	*C. chinense*	H. Hobbs[a]	1992
PI 159236			1992
I-20(AMA12)		AVRDC[b]	1993
Casca Dura Ikeda	*C. annuum*		1993
Yolo Wonder		PetoSeed Company	1992
VR2			1992
VR4			1992
Agronomico 10C-5			1992
Delray Bell		A. Cook[c]	1992
Jaloro		B.Villalon[d]	1993
Magda		Rogers NK Seed Company	1993
Tabasco	*C. frutescens*	McIlhenny Company	1992
Greenleaf Tabasco			1992

[a]Louisiana State University.
[b]Asian Vegetable Research and Development Center.
[c]University of Florida.
[d]Texas A & M University.

Detection of nuclear inclusions. Fifty-four days after inoculation, symptomatic leaves of Yolo Wonder pepper were used to detect nuclear inclusions induced by 10 isolates of TEV. For each isolate, 15 leaves of different infected plants were examined to determine the shape, size and the number of nuclear inclusions. Isolates used for this study included LMS-M, H93-5, C1, MEX-21, TX-M, VIL, CAY-90, 401,V92-4, and DR93-28, the same isolates used in Phase 2 and 3. Epidermal tissues were obtained by peeling the lower surface of the leaf with tweezers as described by Christie and Edwardson (1977). The epidermal tissues were immersed for 5 min in Triton X-100 to dissolve plastids. Strips were submersed in the O-G stain (Calcomine Orange 2RS, Luxol Brilliant Green). Samples were heated in a microwave for 15 sec. The excess O-G stain was removed by rinsing (2-4 times, 30 sec each) with 95% ethanol. Strips were mounted on glass slides using Euparal green, covered with a coverslip and examined with a light microscope using 1000X magnification and an oil immersion objective.

Extraction and analysis of ds RNA. In order to determine if differences exist in dsRNA profiles of TEV isolates, dsRNA were extracted from TEV-infected datura. Isolates used in this study included TEV-401, TEV-DR93-28, TEV-C1, TEV-LMS-M, TEV-CAY-90, TEV-TX-M, TEV-MEX-21, TEV-VIL, TEV-V92-4, and TEV-H93-5, the same isolates used in Phase 2 and 3. Four, 3.5 g samples of leaf tissue were collected from infected datura plants for each isolate. Healthy datura leaves were included as controls. DsRNA was extracted as described by Valverde et al. (1990), with some

modifications. Leaf tissues were squeezed through a leaf roller grinder with 8 ml of 2 x STE buffer (STE buffer =0.001 M ethylenediamine tetraacetic acid, 0.1 M Nacl, and 0.05 M Tris-HCL, pH 6.8) per 3. 5 g sample. The extract was collected in a 50 ml centrifuge tube and 0.5 ml of 2% aqueous bentonite suspension, 1 ml of 10% (SDS) sodium dodecyl sulphate, and 10 ml of 1 x STE saturated phenol were added. Tubes were shaken for 30 min and centrifuged at 8000 g for 15 min at 10 C using a RC 5B Sorvall centrifuge (Du Pont Co., Wilmington, DE.) with a SS-34 rotor. The upper aqueous phase was collected (10 ml) and 2.1 ml of 95% ethanol was added to each tube. Samples were stored overnight at 4 C and subjected to one cycle of fibrous cellulose column chromatography. After washing columns with 100 ml of 1 x STE containing 16% ethanol, the dsRNA was eluted with 6.0 ml of 1 x STE. DsRNA was precipitated at -20 C overnight by adding 0.5 ml of 3 M sodium acetate, pH 5.5, and 20 ml of 95% ethanol. Tubes containing dsRNA were centrifuged for 30 min at 8,000 g and pellets dried at room temperature. DsRNA pellets were resuspended in 200 ul of DNase buffer (0.1 M Tris-HCl, 0.2 M NaCl, 0.01 M $MgCl_2$, pH 7.3). Ten microliters of DNase were added (1 ug/1 ul) to each sample and incubated for 15 min at room temperature. Samples were ethanol precipitated with 5 ul of 3 M sodium acetate and 700 ul of 95% ethanol and centrifuged at 14,000 g for 10 min. Pellets were air-dried at room temperature. One of the four dsRNA pellets of each TEV isolate was resuspended in 300 ul of electrophoresis buffer (0.04 M Tris-HCL, 0.001 M EDTA, and 0.02 M sodium acetate, pH 7.8) that contained 50% of glycerol and 0.01%

bromophenol blue. The dsRNA suspension was serially transferred to the other three tubes containing a dsRNA pellet of the same TEV isolate in order to obtain a concentrated sample. DsRNA extracted from healthy datura was treated similarly. Samples of 60 ul were electrophoresed in a 6% polyacrylamide gel (8 x 10 cm x 1.5 mm) for 3.5 h at 100 V. Gel was stained with ethidium bromide (50 ng/ ml) for 15 min, visualized with a UV light transilluminator (300 nm), and photographed using Polaroid film type 667.

RESULTS

Phase 1

The reaction of 13 pepper lines to 36 TEV isolates are shown in Table 5. Symptoms (MM, M, SM, and SMD) were listed as "S" and symptomless reactions as "NS". Data in Table 5 were arranged to show decreasing frequency of virulence of isolates from top to bottom and increasing frequency of resistance in peppers from left to right. A dendrogram (Rohlf, 1970) based on data from Table 5 showed similar grouping patterns for virus isolates used in Phase 1 (Appendix Figure A2). Serological tests (ELISA) conducted with selected lines that were symptomless or with mild symptoms are presented in Table 6.

Twenty TEV isolates could be grouped according to their ability to induce symptoms on inoculated pepper lines (Table 7). Nevertheless, 16 other isolates could not be grouped due to their unique reactions. Tobacco etch virus isolates 401, C1, CAY-90 and H92-31 induced symptoms on all tested lines (Group 1). In contrast, TEV isolates ATCC-PV-69 and FL-978 (Group 6) induced symptoms only on Casca Dura, Yolo Wonder, and Yolo Y. Groups 2-5 were gradations between these extremes.

Isolates H-93-5 and SEVERE induced symptoms on PI-152225. However, they did not induce symptoms on VR2, which was considered by Greenleaf (1986) to have a lower level of resistance than PI 152225.

Figure 3A shows severe symptoms induced by TEV-H93-5 on PI 152225 and Fig. 3B shows a symptomless VR2 plant after inoculation with the same isolate.

Table 5. Symptoms of 13 pepper lines inoculated with 36 tobacco etch virus isolates in Phase 1

Isolates	Pepper lines				
	Yolo Y Casca Dura Yolo Wonder	Avelar	TF-38A	VR2 136 AA Cook	PI 159236
H92-31, C1, 401, CAY-90	S	S	S	S	S
BUR-TAB	S	S	S	S	S
MEX-21, 15-D	S	S	S	S	S
M-TPC	S	S	S	S	S
NW-M81, LMTP-M GLT-F, VIL, TX-M	S	S	S	S	S
DR93-13, CAJ2A#1 NW-M-83-1 DR92-5, DR93-28	S	S	S	S	S
DR-93-19	S	S	S	S	S
DR-92-7	S	S	S	S	NS
DR-92-6	S	S	NS	S	NS
DR-93-23	S	S	NS	S	NS
H-93-5	S	S	S	NS	S
LMS-M	S	S	NS	S	NS
SEVERE	S	NS	S	NS	S
NW-M-82-2	S	S	S	NS	S
TOM-1	S	NS	S	S	NS
B1, B3	S	S	S	NS	S
C5	S	S	NS	S	NS
C3	S	NS	NS	S	NS
V-92-4	S	S	NS	NS	NS
CAP'86	S	NS	S	NS	S
NW-C86-4	S	NS	NS	NS	NS
ATCC-PV69, FL978	S	NS	NS	NS	NS

S=Symptoms, NS=No symptoms

(table cont'd.)

Isolates	Pepper lines				
	GLT	PI 152225	T.T. MEX'88	Agro.10-C5	Delray Bell
H92-31, C1 401, CAY-90	S	S	S	S	S
BUR-TAB	S	S	S	NS	S
MEX21, 15D	S	S	S	S	NS
M-TPC	S	S	NS	S	NS
NW-M81, LMTP-M GLT-F, VIL, TX-M	S	S	S	NS	NS
DR93-13, CAJ2A#1 NW-M-83-1 DR92-5,DR93-28	NS	NS	NS	S	S
DR-93-19	NS	NS	NS	NS	S
DR-92-7	NS	NS	NS	S	S
DR92-6	S	NS	NS	S	S
DR-93-23	NS	NS	S	S	S
H-93-5	S	S	S	NS	NS
LMS-M	S	NS	NS	S	NS
SEVERE	S	S	S	NS	NS
NW-M82-2	S	NS	S	NS	NS
TOM-1	S	NS	NS	NS	NS
B1, B3	NS	NS	NS	NS	NS
C5	NS	NS	NS	NS	NS
C3	S	NS	NS	NS	NS
V-92-4	S	NS	NS	NS	NS
CAP'86	NS	NS	NS	NS	NS
NW-C-86-4	S	NS	NS	NS	NS
ATCC-PV69, FL978	NS	NS	NS	NS	NS

GLT = Greenleaf Tabasco.
T.T.MEX'88 = Tabasco Type Mex'88.
Agro.10C-5 = Agronomico 10C-5.

Table 6. ELISA results for selected TEV-inoculated pepper lines that showed mild or no symptoms in Phase 1

Isolates	Pepper lines	Symptoms	ELISA
H92-31,CAY-90	PI 152225	MM	+
DR93-28, NW-M83-1, DR92-7, B1	PI 152225	NS	-
15-D, GLT-F, B3	Delray Bell	NS	-
NW-M-81, TX-M, TOM-1, C5	Agronomico 10C-5	NS	-
DR93-13, CAJ2A#1	Tabasco Type Mex'88	NS	-
LMS-M	TF-38A	NS	-
401	Greenleaf Tabasco	MM	+
DR92-5	Greenleaf Tabasco	NS	-
SEVERE	Avelar	NS	-
NW-C86-4	136 A A Cook	NS	-
H93-5, V92-4, ATCC-PV69, CAP'86	VR2	NS	-

MM = Mild mosaic, NS = No symptoms
+ = Positive results.
- = Negative results.

Table 7. Groups of tobacco etch virus isolates that induced similar reactions in 13 pepper lines in Phase 1

	TEV isolates					
Pepper lines	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Casca Dura	S	S	S	S	S	S
Yolo Wonder	S	S	S	S	S	S
Yolo Y	S	S	S	S	S	S
VR2	S	S	S	S	S	NS
136 A A Cook	S	S	S	S	S	NS
Avelar	S	S	S	S	S	NS
Greenleaf Tabasco	S	S	S	S	S	NS
TF-38-A	S	S	S	S	S	NS
PI 159236	S	S	S	S	S	NS
Tabasco Type Mex'88	S	S	NS	S	NS	NS
Agronomico 10C-5	S	S	S	NS	NS	NS
Delray Bell	S	NS	S	NS	NS	NS
PI 152225	S	S	NS	S	NS	NS

Group 1 = isolates H92-31, 401, C1, and CAY-90.
Group 2 = isolates MEX-21 and 15D.
Group 3 = isolates DR92-5, DR93-13, DR93-28, NW-M83-1, and CAJ2A#1.
Group 4 = isolates NW-M81-1, GLT-F, VIL, TX-M, and LMTP-M.
Group 5 = isolates B1 and B3.
Group 6 = isolates ATCC-PV-69 and FL-978.
S = Symptoms, NS = No symptoms.

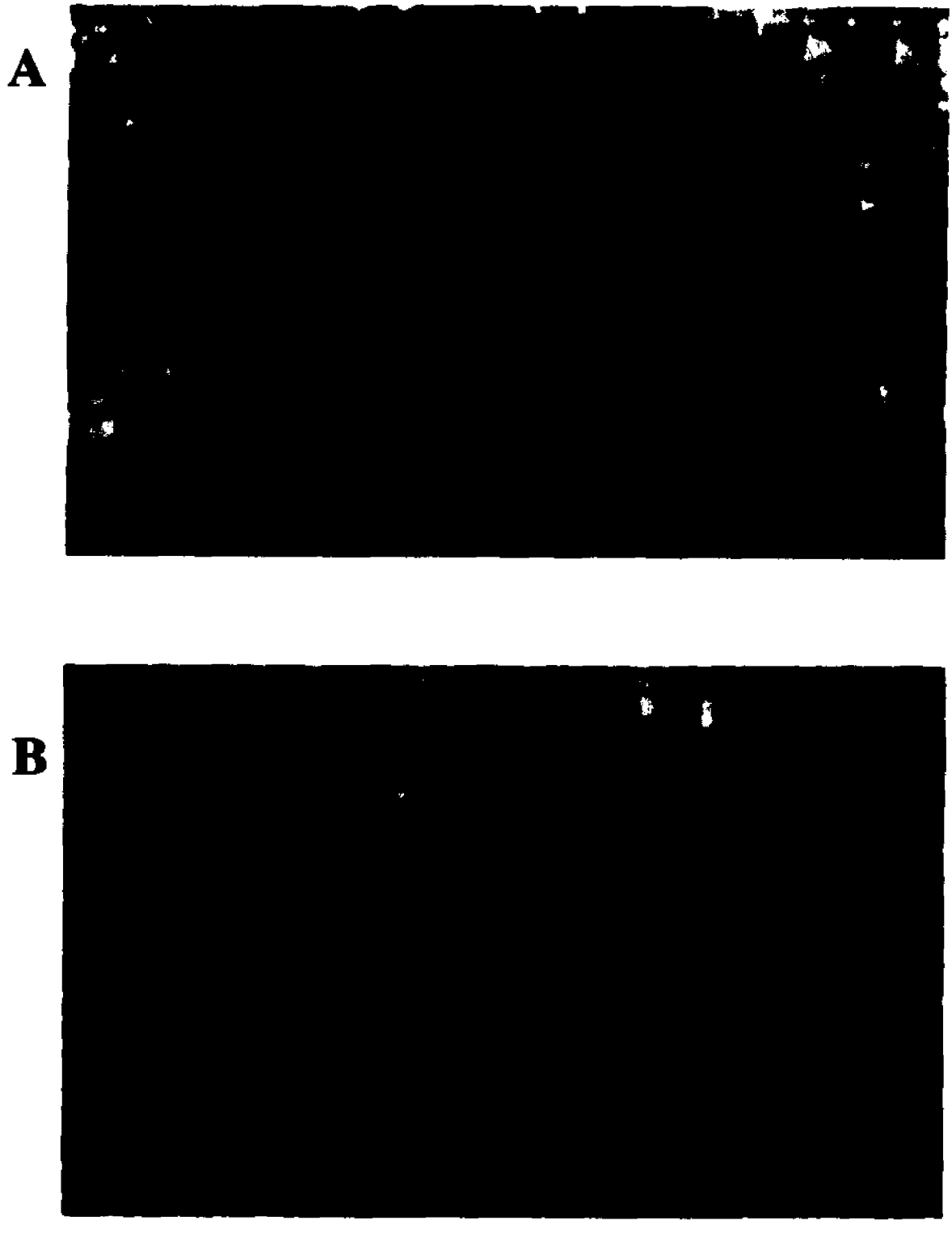


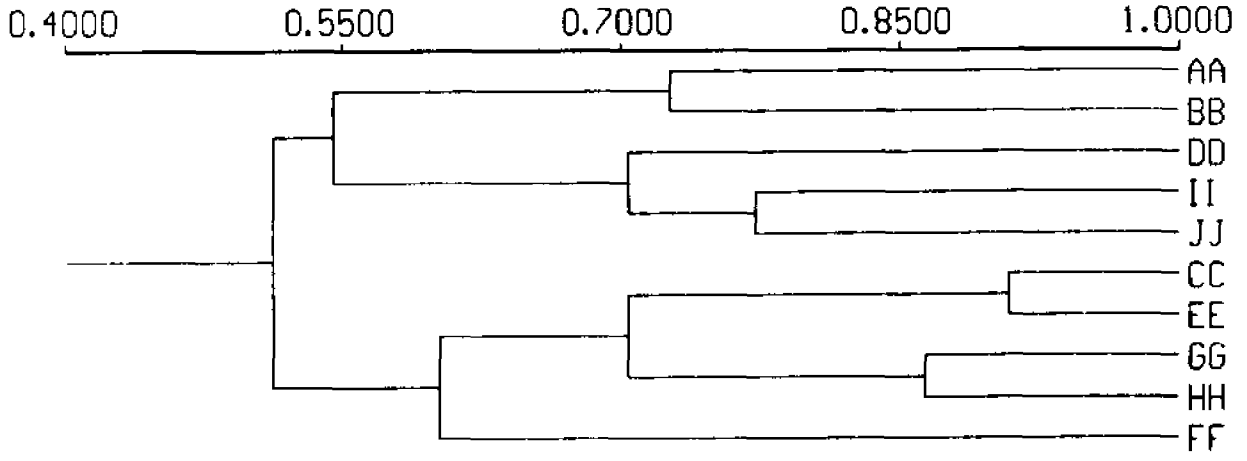
Fig 3. (A) Pepper line PI 152225 showing mosaic symptoms after inoculation with TEV-H93-5. (B) Symptomless VR2 pepper after inoculation with TEV-H93-5.

Figure 4, a dendrogram, illustrates the relationships of TEV reactions of 13 pepper lines used in Phase 1. Pepper lines of the species *C. annuum* clustered together, while a second cluster consisted of pepper lines of *C. frutescens* and *C. chinense*.

Phase 2

Results are shown in Table 8. Pepper lines and isolates were organized to show decreasing virulence of virus isolates from left to right, and increasing resistance of pepper lines from top to bottom. Six of 33 tested pepper lines were susceptible to all 10 TEV isolates. Isolate LMS-M induced severe symptoms in most pepper lines except I-20 and VR4. Isolate V92-4 was the mildest: 17 of 33 pepper lines inoculated with this isolate were symptomless. Only TEV-VIL and TEV-H93-5 were able to induce symptoms on I-20. Mild symptoms were observed on VR4 with TEV- DR93-28, however all the other isolates did not induce symptoms. A dendrogram based on data from Table 8 showed similar grouping patterns for virus isolates used in Phase 2 (Appendix Figure A3). Seven pepper lines obtained from the Plant Genetic Resources Conservation Unit in Georgia showed variable symptoms among the 16 plants inoculated with some TEV isolates. Some plants of individual lines remained symptomless while the other plants of the same line showed symptoms. Apparently, genetic segregation occurred in these lines due to outcrossing.

ELISA results. Some symptomless lines inoculated with TEV were ELISA positive (Table 9), while ELISA tests on others revealed that symptomless plants did not contain virus.



AA- Yolo Y, Casca Dura, YW
BB- Avelar
DD- VR2, 136 A A Cook
II- Agronomico 10C-5
JJ- Delray Bell

Capsicum annuum

CC- TF-38A
EE- PI 159236
GG- PI 152225

Capsicum chinense

HH- Tabasco Type Mex'88
FF- Greenleaf Tabasco

Capsicum frutescens

Fig. 4. A dendrogram of the data in Table 5 showing relationship of TEV reactions and species origin of 13 pepper lines used in Phase 1.

Table 8. Symptoms of 33 pepper lines inoculated with 10 tobacco etch virus isolates

	TEV isolates									
Pepper lines	LMS -M	VIL	DR93 -28	TX -M	401	CAY -90	C1	MEX -21	H93 -5	V92 -4
Yolo Wonder	S	S	S	S	S	S	S	S	S	S
Casca Dura	S	S	S	S	S	S	S	S	S	S
ELS-2-1	S	S	S	S	S	S	S	S	S	S
Veracruz	S	S	S	S	S	S	S	S	S	S
Tam Veracruz	S	S	S	S	S	S	S	S	S	S
S-20-1	S	S	S	S	S	S	S	S	S	S
PI 264281	SEG	SEG	S	SEG	S	SEG	S	SEG	SEG	SEG
Puerto Rico Wonder	S	S	S	S	SEG	SEG	S	SEG	SEG	S
Casca Grossa	S	S	S	S	S	S	SEG	S	SEG	S
Avelar	S	SEG	SEG	SEG	SEG	SEG	SEG	SEG	SEG	SEG
Agronomico 10G24-7	S	SEG	SEG	SEG	SEG	SEG	SEG	SEG	SEG	SEG
Agronomico 8	S	SEG	SEG	SEG	S	S	S	SEG	NS	SEG
Agronomico 10C-5	SEG	SEG	SEG	SEG	SEG	SEG	NS	SEG	NS	SEG
Tam Mild Jalapeno	S	S	S	S	S	NS[c]	S	S	S	S
CO 1664	S	S	S	S	S	NS	S	S	S	S
SC 46252	S	S	S	S	S	S	S	NS	NS	NS
92LB444Q	S	S	S	S	S	S	S	NS	NS	NS
Tsch-2	S	S	S	S	NS	NS	NS	S	S	S
FLBG-1	S	S	S	S	S	NS	NS	S	S	NS
Hidalgo	S	S	NS	S	S	S	NS	S	S	NS
Rio Grande Gold	S	S	S	NS	S	S	S	NS	S	NS
Marquis	S	S	S	S	S	S	S	NS	NS	NS
Elisa	S	S	S	S	S	S	S	NS	NS	NS
King Arthur	S	S	S	S	S	S	S	NS	NS	NS
Bomby	S	S	S	NS	S	S	S	NS	NS	NS
Reinger	S	S	S	NS	S	S	S	NS	NS	NS
Magda	S	S	S	S	S	NS	NS	NS	NS	NS
Tambel-2	S	NS	S	S	NS	NS	NS	S	S	NS
Casca Dura Ikeda	S	S	S	S	NS	NS	NS	NS	NS	NS
LP-1	S	NS	NS	S	NS	NS	NS	NS	NS	NS
I-20	NS	S	NS	NS	NS	NS	NS	NS	S	NS
Jaloro	S	NS	NS	NS	NS	NS	NS	NS	NS	NS
VR4	NS	NS	S	NS	NS	NS	NS	NS	NS	NS

S= Symptoms, SEG=Segregation (genetically heterogeneous due to outcrossing), NS=No symptoms.

Table 9. ELISA results for selected symptomless pepper lines inoculated with 10 tobacco etch virus isolates in Phase 2

Isolates	Pepper lines	ELISA reaction
LMS-M	VR4	+[a]
LMS-M	I-20	-[b]
VIL	Jaloro, Tambel-2	+
DR93-28	Jaloro, LP-1	-
TX-M	Bomby	+
TX-M	VR4	-
401	TSCH-2	+
401	Casca Dura Ikeda	-
CAY-90	Magda	-
CAY-90	I-20	+
C1	Hidalgo	-
C1	I-20	+
MEX-21	SC 46252	-
MEX-21	Casca Dura Ikeda	+
H93-5	Magda, VR4	-
V92-4	King Arthur, LP-1	-

+ = Positive results.
- = Negative results.

Phase 3

Table 10 shows the symptoms of 13 pepper lines inoculated with 10 TEV isolates and the ELISA results. The comparison between ELISA values of the isolate-pepper combination and the negative control (uninoculated pepper) was used as a guideline to determine the presence or absence of virus. If the value was twice the ELISA value of the negative control, the pepper was considered positive. ELISA was not performed with Tabasco pepper due to death of most plants.

Tabasco, Yolo Wonder, and I-20 were susceptible to all TEV isolates tested. In contrast, Jaloro, VR4, Delray Bell, and Agronomico 10C-5 were resistant to many TEV isolates tested. Agronomico 10C-5 and Delray Bell showed similar symptoms with all 10 TEV isolates. Likewise, Magda and Casca Dura Ikeda behaved similarly. Isolates 401 and DR93-28 induced symptoms in all the peppers tested. Most plants with symptoms were ELISA positive and most symptomless plants were ELISA negative. But some symptomless plants were ELISA positive and some plants with symptoms were ELISA negative. Isolate-pepper line reactions sometimes differed between experiments 1 and 2 with respect to symptoms and / or ELISA. Isolate LMS -M induced mild mosaic in Delray Bell and Agronomico 10C-5, but it induced severe symptoms in all the other infected pepper lines (Fig. 5). A dendrogram based on data from Table 10 showed similar grouping patterns for virus isolates used in Phase 3 (Appendix Figure A 4).

Table 11 lists TEV symptom reactions of pepper lines used in Phase 3. The more severe symptom evaluation of either experiment 1 or experiment 2 was listed in the table.

Table 10. Symptoms and ELISA results of 13 pepper lines inoculated with 10 TEV isolates in Phase 3. Data reflects the results of two experiments

Pepper lines		Isolates					
		401		DR93-28		TX-M	
	Expt[a]	Symptom[b]	ELISA[c]	Symptom	ELISA	Symptom	ELISA
Tabasco	1	W	NT	W	NT	W	NT
	2	M,W	NT	W	NT	M,S	NT
Yolo Wonder	1	SM	+	SMD[h]	+	SM	+
	2	SMD	+	SMD	+	SM,M	+
I-20	1	SM	+	SMD	+	SM	+
	2	SM,M	+	SMD,SM	+	MM	+
Greenleaf Tabasco	1	M,W	+	NS,W	+	M,W	+
	2	M,S	+	M,MM	+	M	+
PI 159236	1	M,SM	+	M	+	M	+
	2	SM	+	SM	+	M	+
Jaloro	1	NS	+	NS	+	MM	+
	2	MM	+	MM	-	MM	+
Magda	1	M	+	M	+	MM	+
	2	M	+	M	+	M,MM	+
Casca Dura Ikeda	1	M	+	M	+	MM	+
	2	M	+	SM,M	+	MM	+
PI 152225	1	M	+	NS	+	M,MM	+
	2	M	+	MM	-	M,MM	+
VR2	1	M	+	SMD	+	M	+
	2	SM	+	SMD	+	M,MM	+
VR4	1	MM	+	M	+	NS	-
	2	M	+	M	+	NS	-
Delray Bell	1	MM	-	M,MM	+	NS	-
	2	M,MM	+	MM	+	NS	-
Agronomico 10C-5	1	M	+	M,MM	+	NS	-
	2	M,MM	+	MM	+	NS	-

[a]Experiments. *(table cont'd)*

[b]W = Wilt, NT = Not tested, S = Stunt, NS = No symptoms, MM = Mild mosaic, M = Mosaic, SM = Severe mosaic, SMD = Severe mosaic and leaf distortion.

[c]+ = Positive results, - = Negative results.

Pepper lines		Isolates					
		VIL		MEX-21		LMS-M	
	Expt	Symptom	ELISA	Symptom	ELISA	Symptom	ELISA
Tabasco	1	W	NT	W	NT	W	NT
	2	M,S	NT	M,S,W	NT	M,S,W	NT
Yolo Wonder	1	M	+	SM	+	SMD	+
	2	M	+	SMD,SM	+	SMD,S	+
I-20	1	M	+	SM	+	SMD,M	+
	2	MM	+	SM,M	+	M	-
Greenleaf Tabasco	1	M,S	+	M,W	+	NS,W	-
	2	M	+	M,MM	+	NS	-
PI 159236	1	M	+	M,MM	+	NS	-
	2	MM	+	M,MM	+	NS	-
Jaloro	1	NS	+	MM	+	SM,M	+
	2	NS	+	MM	+	SM,M	+
Magda	1	MM	+	MM	+	SMD	+
	2	MM	-	M,MM	+	SM,M	+
Casca Dura Ikeda	1	MM	+	MM	+	SMD	+
	2	MM	-	MM	+	SM,M	+
PI 152225	1	M	+	M,MM	+	NS	-
	2	MM	+	MM	+	NS	-
VR2	1	M	+	NS	-	SMD	+
	2	M	+	NS	+	SMD,S	+
VR4	1	NS	+	NS	-	SMD,M	+
	2	NS	-	NS	-	SM	+
Delray Bell	1	NS	-	NS	-	M,MM	-
	2	NS	-	NS	-	M,MM	-
Agronomico 10C-5	1	NS	-	NS	-	M,MM	+
	2	NS	-	NS	-	MM	+

(table cont'd)

Pepper lines	Isolates				
		CAY-90		C1	
	Expt	Symptom	ELISA	Symptom	ELISA
Tabasco	1	W	NT	W	NT
	2	M,S	NT	W	NT
Yolo Wonder	1	SM,M	+	M	+
	2	M	+	SMD,S	+
I-20	1	M	+	SM	+
	2	NS	+	SMD,SM	+
Greenleaf Tabasco	1	M,W	+	NS,W	-
	2	NS	-	M,S	-
PI 159236	1	MM	+	MM	+
	2	MM	-	SM,M	+
Jaloro	1	NS	-	NS	-
	2	NS	-	NS	-
Magda	1	MM	+	NS	-
	2	MM	-	NS	-
Casca Dura Ikeda	1	MM	+	NS	-
	2	MM	-	NS	-
PI 152225	1	NS	-	MM	+
	2	NS	-	M,MM	-
VR2	1	M	+	SM	+
	2	M	+	SMD	+
VR4	1	MM	+	NS	+
	2	NS	-	M	-
Delray Bell	1	NS	+	NS	-
	2	NS	-	MM	-
Agronomico 10C-5	1	NS	+	MM	+
	2	NS	-	MM	-

(table cont'd)

Pepper lines	Isolates				
		H93-5		V92-4	
	Expt	Symptom	ELISA	Symptom	ELISA
Tabasco	1	W	NT	S,W	NT
	2	M,S,W	NT	SMD,S	NT
Yolo Wonder	1	SMD	+	M	+
	2	SMD	+	M	+
I-20	1	SM	+	SMD,M	+
	2	SMD,SM	+	SMD,SM	+
Greenleaf Tabasco	1	SMD,M	+	M	+
	2	SMD,S	+	MM	-
PI 159236	1	SMD	+	MM	+
	2	SMD	+	MM	+
Jaloro	1	NS	-	NS	-
	2	NS	-	NS	-
Magda	1	NS	-	NS	-
	2	NS	-	NS	-
Casca Dura Ikeda	1	NS	-	NS	-
	2	NS	-	NS	-
PI 152225	1	SM	+	NS	-
	2	SM	+	NS	-
VR2	1	NS	-	NS	-
	2	NS	-	NS	-
VR4	1	NS	-	NS	-
	2	NS	-	NS	-
Delray Bell	1	NS	-	NS	-
	2	NS	-	NS	-
Agronomico10C-5	1	NS	-	NS	-
	2	NS	-	NS	-

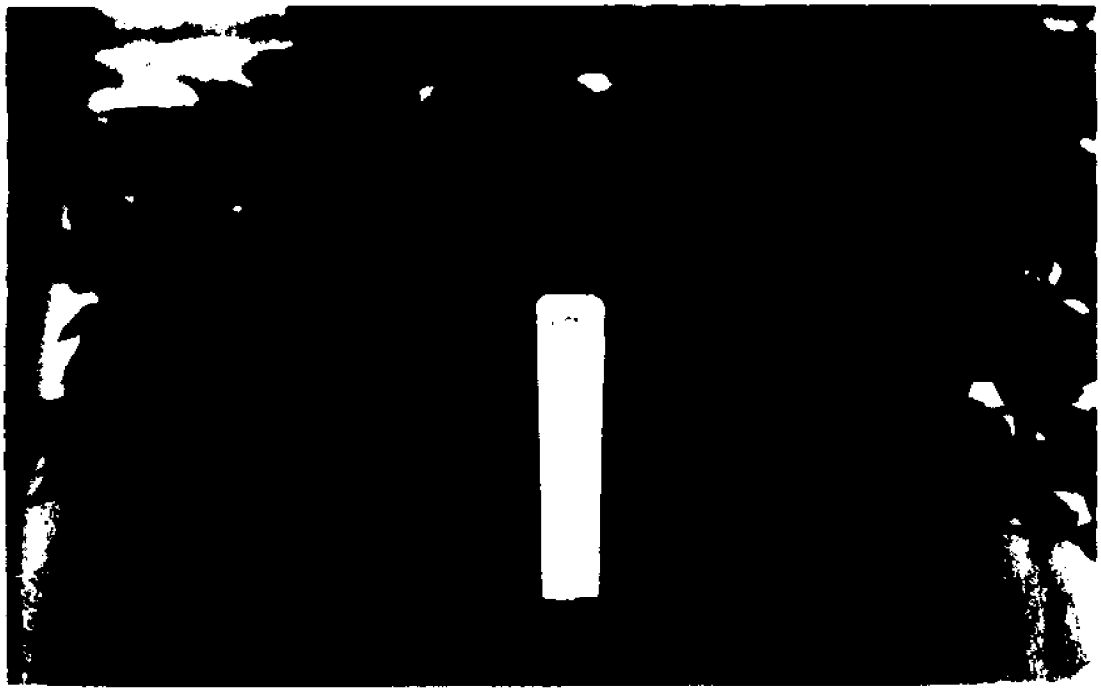


Fig 5. Tobacco etch virus isolate LMS-M inducing severe symptoms on four pepper lines.

Table 11. Summary of tobacco etch virus isolate reactions in pepper lines in Phase 3, using the more susceptible reaction of either experiment 1 or experiment 2

	Isolates									
Pepper lines	401	DR93 -28	LMS -M	TX -M	C1	CAY -90	VIL	MEX -21	H93 -5	V92 -4
Tabasco	W[a]	W	W	W	W	W	W	W	W	W
Yolo Wonder	SMD[b]	SMD	SMD	SM[c]	SMD	SM	M[d]	SMD	SMD	M
I-20	SM	SMD	SMD	SM	SMD	M	M	SM	SMD	SMD
GLT[e]	M	M	NS[f]	M	M	M	M	M,MM	SMD	M
PI 159236	SM	SM	NS	M	SM	MM	M	M	SMD	MM
Jaloro	MM	MM	SM	MM	NS	NS	NS	MM	NS	NS
Magda	M	M	SMD	M	NS	MM	MM	M	NS	NS
C. D. Ikeda[g]	M	M	SMD	MM	NS	MM	MM	MM	NS	NS
PI 152225	M	MM	NS	M	M	NS	M	M	SM	NS
VR2	SM	SMD	SMD	M	SM	M	M	NS	NS	NS
VR4	M	M	SMD	NS	M	MM	NS	NS	NS	NS
Delray Bell	M	M	M	NS	MM	NS	NS	NS	NS	NS
Agro.10C-5[h]	M	M	M	NS	MM	NS	NS	NS	NS	NS

[a]W = Wilt.
[b]SMD = Severe mosaic and leaf distortion.
[c]SM = Severe mosaic.
[d]M = Mosaic.
[e]GLT = Greenleaf Tabasco.
[f]NS = No symptoms.
[g]C. D. Ikeda = Casca Dura Ikeda.
[h]Agro.10C-5 = Agronomico 10C-5.

Statistical analysis. Tables 12 through 21 contain results of statistical analysis of mean ELISA absorbance of individual isolates in 12 pepper lines. Combined analysis of results of experiments 1 and 2 are also included.

Table 22 shows the ELISA results for 10 isolates of TEV inoculated to 13 peppers tested in Phase 3. The data reflects the combined analysis of experiments 1 and 2.

Nuclear inclusions induced by different isolates of TEV. The nuclear inclusions observed in Yolo Wonder infected with several isolates of TEV are shown in Figure 6. Inclusions were green after O-G staining. Nuclei stained light brown. Isolate TEV-401 induced square-shaped inclusions (Fig. 6B). Inclusions induced by TEV-DR93-28, TEV-V92-4, and TEV-C1 also were square in shape. Isolate TX-M induced large, square inclusions that were seen in most nuclei. Nuclear inclusions of TEV-CAY-90 consisted of folded plates (Fig. 6C). Some inclusions induced by TEV-MEX-21 and TEV-VIL were seen as curved plates (Fig. 6D), but others were seen as square plates. Needle-like inclusions were observed in TEV-LMS-M infected cells. Inclusions induced by this TEV isolate were seen as a group of crystals forming a star-like structure (Fig. 6E). Isolate H93-5 induced several rectangular inclusions per nuclei. In some cases, there were as many as four inclusions in one nucleus (Fig. 6F). Inclusions were commonly seen in nuclei of guard cells. In general, the type of inclusions associated with one or more TEV isolates were consistently found in examined samples.

Table 12. Comparison of symptoms and ELISA light absorbance values for leaf extracts from 12 pepper lines inoculated with TEV-401 obtained from two repeated experiments in Phase 3[u]

	Experiment 1		Experiment 2		Experiment 1 & 2[v]	
Pepper lines	ELISA[w]	Symptom[x]	ELISA[w]	Symptom[x]	ELISA[y]	Healthy[z]
PI 159236	1.398^{ab}	M,SM	1.464^{a}	SM	1.431^{a}	0.157
VR2	1.411^{ab}	M	1.116^{ab}	SM	1.263^{ab}	0.164
Greenleaf Tabasco	1.473^{a}	M,W	0.612^{cde}	M,S	1.043^{bc}	0.129
PI 152225	0.971^{cd}	M	0.965^{bc}	M	0.968^{c}	0.158
I-20	1.106^{bc}	SM	0.677^{cd}	SM,M	0.892^{c}	0.144
Yolo Wonder	0.814^{cde}	SM	0.912^{bc}	SMD	0.863^{c}	0.170
Magda	0.750^{de}	M	0.403^{def}	M	0.567^{d}	0.149
Casca Dura Ikeda	0.693^{de}	M	0.318^{def}	M	0.505^{d}	0.139
VR4	0.645^{de}	MM	0.297^{def}	M	0.471^{d}	0.172
Agronomico10C-5	0.608^{e}	M	0.229^{ef}	M,MM	0.418^{d}	0.124
Jaloro	0.514^{e}	NS	0.281^{def}	MM	0.398^{d}	0.147
Delray Bell	0.517^{e}	MM	0.145^{f}	M,MM	0.331^{d}	0.141

[u]General Linear Models Procedure; Tukey's Studentized Range (HSD) Test was used to compare ELISA absorbance value (405 nm) from different pepper lines and cultivars. Values that share the same letters are not significantly different (p=0.05) from each other.
[v]Combined analysis of mean ELISA absorbance of experiments 1 and 2.
[w]Mean ELISA absorbance of 4 samples.
[x]Symptom: M=Mosaic, SM=Severe mosaic, W=Wilt, S=Stunt, SMD=Severe mosaic and leaf distortion, MM=Mild mosaic.
[y]Mean ELISA absorbance of 8 inoculated pepper samples.
[z]Mean ELISA absorbance of 8 healthy pepper samples.

Table 13. Comparison of symptoms and ELISA light absorbance values for leaf extracts from 12 pepper lines inoculated with TEV-DR93-28 obtained from two repeated experiments in Phase 3[u]

	Experiment 1		Experiment 2		Experiment 1 & 2[v]	
Pepper lines	ELISA[w]	Symptom[x]	ELISA[w]	Symptom[x]	ELISA[y]	Healthy[z]
VR2	1.080[a]	SMD	0.999[a]	SMD	1.040[a]	0.118
Yolo Wonder	0.908[ab]	SMD	0.595[abc]	SMD	0.752[ab]	0.112
PI 159236	0.589[bc]	M	0.727[ab]	SM	0.658[bc]	0.108
I-20	0.440[c]	SMD	0.569[abc]	SMD,SM	0.505[bcd]	0.080
VR4	0.541[c]	M	0.378[bc]	M	0.459[bcd]	0.108
Casca Dura Ikeda	0.620[bc]	M	0.284[bc]	SM,M	0.452[bcd]	0.088
Magda	0.556[bc]	M	0.244[bc]	M	0.400[cd]	0.089
Delray Bell	0.444[c]	M,MM	0.168[c]	MM	0.306[d]	0.085
Agronomico10C-5	0.352[c]	M,MM	0.257[bc]	MM	0.305[d]	0.073
Jaloro	0.457[c]	NS	0.103[c]	MM	0.280[d]	0.104
PI 152225	0.297[c]	NS	0.141[c]	MM	0.219[d]	0.097
Greenleaf Tabasco	0.283[c]	NS,W	0.141[c]	M,MM	0.212[d]	0.084

[u]General Linear Models Procedure; Tukey's Studentized Range (HSD) Test was used to compare ELISA absorbance value (405 nm) from different pepper lines and cultivars. Values that share the same letters are not significantly different (p=0.05) from each other.
[v]Combined analysis of mean ELISA absorbance of experiments 1 and 2.
[w]Mean ELISA absorbance of 4 samples.
[x]Symptom: SMD=Severe mosaic and leaf distortion, M=Mosaic, SM=Severe mosaic, NS=No symptoms, W=Wilt.
[y]Mean ELISA absorbance of 8 inoculated pepper samples.
[z]Mean ELISA absorbance of 8 healthy pepper samples.

Table 14. Comparison of symptoms and ELISA light absorbance values for leaf extracts from 12 pepper lines inoculated with TEV-VIL obtained from two repeated experiments in Phase 3[u]

	Experiment 1		Experiment 2		Experiment 1 & 2[v]	
Pepper lines	ELISA[w]	Symptom[x]	ELISA[w]	Symptom[x]	ELISA[y]	Healthy[z]
I-20	1.105[ab]	M	1.402[a]	MM	1.254[a]	0.124
PI 159236	1.274[a]	M	1.119[ab]	MM	1.197[a]	0.122
Greenleaf Tabasco	0.965[ab]	M,S	1.358[ab]	M	1.162[a]	0.128
VR2	1.105[ab]	M	0.964[b]	M	1.034[a]	0.126
Yolo Wonder	0.942[ab]	M	0.519[c]	M	0.730[b]	0.119
PI 152225	0.845[bc]	M	0.527[c]	MM	0.686[b]	0.114
Casca Dura Ikeda	1.154[ab]	MM	0.090[d]	MM	0.621[b]	0.108
Magda	1.161[ab]	MM	0.074[d]	MM	0.617[b]	0.119
Jaloro	0.995[ab]	NS	0.237[cd]	NS	0.616[b]	0.111
VR4	0.503[cd]	NS	0.353[b]	NS	0.269[c]	0.116
Agronomico10-C-5	0.196[d]	NS	0.080[d]	NS	0.138[c]	0.122
Delray Bell	0.213[d]	NS	0.051[d]	NS	0.132[c]	0.116

[u]General Linear Models Procedure; Tukey's Studentized Range (HSD) Test was used to compare ELISA absorbance value (405 nm) from different pepper lines and cultivars. Values that share the same letters are not significantly different (p=0.05) from each other.
[v]Combined analysis of mean ELISA absorbance of experiments 1 and 2.
[w]Mean ELISA absorbance of 4 samples.
[x]Symptom: M=Mosaic, MM=Mild mosaic, S=Stunt, NS=No symptoms.
[y]Mean ELISA absorbance of 8 inoculated pepper samples.
[z]Mean ELISA absorbance of 8 healthy pepper samples.

Table 15. Comparison of symptoms and ELISA light absorbance values for leaf extracts from 12 pepper lines inoculated with TEV-CAY-90 obtained from two repeated experiments in Phase 3[u]

	Experiment 1		Experiment 2		Experiment 1 & 2[v]	
Pepper lines	ELISA[w]	Symptom[x]	ELISA[w]	Symptom[x]	ELISA[y]	Healthy[z]
VR2	1.444[a]	M	0.597[a]	M	1.020[a]	0.166
YW	0.825[b]	SM,M	0.644[a]	M	0.735[b]	0.149
I-20	0.595[bc]	M	0.178[bc]	NS	0.387[c]	0.155
Casca Dura Ikeda	0.553[bcd]	MM	0.120[bc]	MM	0.336[cd]	0.127
VR4	0.482[bcd]	MM	0.152[bc]	NS	0.317[cd]	0.147
Magda	0.451[cd]	MM	0.095[c]	MM	0.272[cd]	0.151
PI 159236	0.327[cd]	MM	0.216[b]	MM	0.271[cd]	0.153
Delray Bell	0.359[cd]	NS	0.120[bc]	NS	0.239[cd]	0.127
Agronomico10C-5	0.372[cd]	NS	0.083[c]	NS	0.227[cd]	0.146
Greenleaf Tabasco	0.325[cd]	M,W	0.098[bc]	NS	0.213[cd]	0.142
Jaloro	0.226[d]	NS	0.124[bc]	NS	0.175[d]	0.130
PI 152225	0.190[d]	NS	0.128[c]	NS	0.159[d]	0.142

[u]General Linear Models Procedure; Tukey's Studentized Range (HSD) Test was used to compare ELISA absorbance value (405 nm) from different pepper lines and cultivars. Values that share the same latters are not significantly different (p=0.05) from each other.
[v]Combined analysis of mean ELISA absorbance of experiments 1 and 2.
[w]Mean ELISA absorbance of 4 samples.
[x]Symptom: M=Mosaic, SM=Severe mosaic, NS=No symptoms, MM=Mild mosaic, W=Wilt.
[y]Mean ELISA absorbance of 8 inoculated pepper samples.
[z]Mean ELISA absorbance of 8 healthy pepper samples.

Table 16. Comparison of symptoms and ELISA light absorbance values for leaf extracts from 12 pepper lines inoculated with TEV-C1 obtained from two repeated experiments in Phase 3[u]

	Experiment 1		Experiment 2		Experiment 1 & 2[v]	
Pepper lines	ELISA[w]	Symptom[x]	ELISA[w]	Symptom[x]	ELISA[y]	Healthy[z]
I-20	1.134[ab]	SM	1.149[a]	SMD,SM	1.142[a]	0.123
VR2	1.464[a]	SM	0.550[bc]	SMD	1.001[a]	0.151
Yolo Wonder	0.535[c]	M	0.951[ab]	SMD,S	0.743[ab]	0.139
PI 159236	0.893[b]	MM	0.198[c]	SM,M	0.546[bc]	0.133
PI 152225	0.453[cd]	MM	0.142[c]	M,MM	0.298[bc]	0.129
VR4	0.488[cd]	NS	0.094[c]	M	0.291[bc]	0.143
Agronomico10C-5	0.456[cd]	MM	0.092[c]	MM	0.274[c]	0.115
Greenleaf Tabasco	0.302[cd]	NS,W	0.140[c]	M,S	0.221[c]	0.121
Delray Bell	0.285[cd]	NS	0.105[c]	MM	0.195[c]	0.117
Jaloro	0.236[cd]	NS	0.046[c]	NS	0.141[c]	0.131
Magda	0.179[d]	NS	0.079[c]	NS	0.129[c]	0.129
Casca Dura Ikeda	0.203[d]	NS	0.048[c]	NS	0.125[c]	0.115

[u]General Linear Models Procedure; Tukey's Studentized Range (HSD) Test was used to compare ELISA absorbance value (405 nm) from different pepper lines and cultivars. Values that share the same letters are not significantly different (p=0.05) from each other.
[v]Combined analysis of mean ELISA absorbance of experiments 1 and 2.
[w]Mean ELISA absorbance of 4 samples.
[x]Symptom: SM=Severe mosaic, SMD=Severe mosaic and leaf distortion, M=Mosaic, S=Stunt, MM=Mild mosaic, NS=No symptoms, W=Wilted.
[y]Mean ELISA absorbance of 8 inoculated pepper samples.
[z]Mean ELISA absorbance of 8 healthy pepper samples.

Table 17. Comparison of symptoms and ELISA light absorbance values for leaf extracts from 12 pepper lines inoculated with TEV-TX-M obtained from two repeated experiments in Phase 3[u]

	Experiment 1		Experiment 2		Experiment 1& 2[v]	
Pepper lines	ELISA[w]	Symptom[x]	ELISA[w]	Symptom[x]	ELISA[y]	Healthy[z]
Jaloro	1.192^{ab}	MM	0.846^{ab}	MM	1.019^{a}	0.098
PI 152225	1.471^{a}	M,MM	0.382^{d}	M,MM	0.926^{ab}	0.099
Greenleaf Tabasco	1.128^{ab}	M,W	0.702^{bc}	M	0.915^{ab}	0.085
PI 159236	0.780^{bc}	M	1.005^{a}	M	0.892a^{b}	0.087
VR2	1.100^{ab}	M	0.555^{cd}	M,MM	0.827^{ab}	0.097
Casca Dura Ikeda	1.114^{ab}	MM	0.412^{d}	MM	0.763^{abc}	0.010
Magda	0.938^{ab}	MM	0.423^{d}	M,MM	0.681^{bc}	0.084
I-20	0.844^{b}	SM	0.441^{d}	MM	0.643^{bc}	0.081
Yolo Wonder	0.673^{bcd}	SM	0.342^{d}	SM,M	0.507^{c}	0.100
Delray Bell	0.207^{cd}	NS	0.049^{e}	NS	0.128^{d}	0.101
Agronomico10C-5	0.157^{d}	NS	0.060^{e}	NS	0.109^{d}	0.082
VR4	0.143^{d}	NS	0.059^{e}	NS	0.101^{d}	0.100

[u]General Linear Models Procedure; Tukey's Studentized Range (HSD) Test was used to compare ELISA absorbance value (405 nm) from different pepper lines and cultivars. Values that share the same letters are not significantly different (p=0.05) from each other.
[v]Combined analysis of ELISA absorbance of experiments 1 and 2.
[w]Mean ELISA absorbance of 4 samples.
[x]Symptom: M=Mosaic, MM=Mild mosaic, W=Wilted, SM=Severe Mosaic, NS=No symptoms.
[y]Mean ELISA absorbance of 8 inoculated pepper samples.
[z]Mean ELISA absorbance of 8 healthy pepper samples.

Table 18. Comparison of symptoms and ELISA light absorbance values for leaf extracts from 12 pepper lines inoculated with TEV-LMS-M, obtained from two repeated experiments in Phase 3[u]

	Experiment 1		Experiment 2		Experiment 1 & 2[v]	
Pepper lines	ELISA[w]	Symptom[x]	ELISA[w]	Symptom[x]	ELISA[y]	Healthy[z]
VR2	1.479[a]	SMD	0.265[bc]	SMD,S	0.981[a]	0.127
Yolo Wonder	0.831[abc]	SMD	0.348[ab]	SMD,S	0.737[ab]	0.143
Jaloro	0.717[bc]	SM,M	0.357[a]	SM,M	0.683[abc]	0.139
Magda	0.745[abc]	SMD	0.242[c]	SM,M	0.600[bcd]	0.111
I-20	0.961[ab]	SMD,M	0.079[d]	M	0.541[bcde]	0.114
Casca Dura Ikeda	0.732[abc]	SMD	0.094[d]	SM,M	0.437[bcdef]	0.127
VR4	0.567[bc]	SMD,M	0.090[d]	SM	0.348[cdef]	0.139
Agronomico10C-5	0.445[bc]	M,MM	0.078[d]	MM	0.282[def]	0.115
Delray Bell	0.387[bc]	M,MM	0.044[d]	M,MM	0.219[ef]	0.130
PI 159236	0.252[bc]	NS	0.041[d]	NS	0.150[f]	0.113
Greenleaf Tabasco	0.218[bc]	NS,W	0.039[d]	NS	0.133[f]	0.114
PI 152225	0.195[c]	NS	0.040[d]	NS	0.114[f]	0.136

[u]General Linear Models Procedure; Tukey's Studentized Range (HSD) Test was used to compare ELISA absorbance value (405 nm) from different pepper lines and cultivars. Values that share the same letters are not significantly different (p=0.05) from each other.
[v]Combined analysis of mean ELISA absorbance of experiments 1 and 2.
[w]Mean ELISA absorbance of 4 samples.
[x]Symptom: SMD=Severe mosaic and leaf distortion, S=Stunt, SM=Severe mosaic, M=Mosaic, MM=Mild mosaic, NS=No symptoms, W=Wilt.
[y]Mean ELISA absorbance of 8 inoculated pepper samples.
[z]Mean ELISA absorbance of 8 healthy pepper samples.

Table 19. Comparison of symptoms and ELISA light absorbance values for leaf extracts from 12 pepper lines inoculated with TEV-MEX-21 obtained from two repeated experiments in Phase 3[u]

	Experiment 1		Experiment 2		Experiment 1 & 2[v]	
Pepper lines	ELISA[w]	Symptom[x]	ELISA[w]	Symptom[x]	ELISA[y]	Healthy[z]
I-20	1.467[a]	SM	1.320[ab]	SM,M	1.394[a]	0.129
PI 159236	1.020[ab]	M,MM	1.710[a]	M,MM	1.365[ab]	0.136
Greenleaf Tabasco	1.390[a]	M,W	0.879[bcd]	M,MM	1.134[abc]	0.121
Jaloro	0.919[ab]	MM	0.954[bc]	MM	0.936[abc]	0.133
Yolo Wonder	1.469[a]	SM	0.345[cde]	SMD,SM	0.907[bcd]	0.147
PI 152225	0.555[bc]	M,MM	0.997[abc]	MM	0.776[cde]	0.146
Magda	0.491[bc]	MM	0.599[bcde]	M,MM	0.545[def]	0.133
Casca Dura Ikeda	0.503[bc]	MM	0.521[cde]	MM	0.512[def]	0.131
VR2	0.147[c]	NS	0.565[cde]	NS	0.356[ef]	0.140
VR4	0.194[c]	NS	0.168[de]	NS	0.181[f]	0.145
Agronomico10C-5	0.149[c]	NS	0.123[e]	NS	0.136[f]	0.127
Delray Bell	0.094[c]	NS	0.145[de]	NS	0.120[f]	0.121

[u]General Linear Models Procedure; Tukey's Studentized Range (HSD) Test was used to compare ELISA absorbance value (405 nm) from different pepper lines and cultivars. Values that share the same letters are not significantly different (p=0.05) from each other.
[v]Combined analysis of mean ELISA absorbance of experiments 1 and 2.
[w]Mean ELISA absorbance of 4 samples.
[x]Symptom: SM=Severe mosaic, M=Mosaic, MM=Mild mosaic, W=Wilt, SMD=Severe mosaic and leaf distortion, NS=No symptoms.
[y]Mean ELISA absorbance of 8 inoculated pepper samples.
[z]Mean ELISA absorbance of 8 healthy pepper samples.

Table 20. Comparison of symptoms and ELISA light absorbance values for leaf extracts from 12 pepper lines inoculated with TEV-V92-4 obtained from two repeated experiments in Phase 3[u]

	Experiment-1		Experiment-2		Experiment 1 & 2[v]	
Pepper lines	ELISA[w]	Symptom[x]	ELISA[w]	Symptom[x]	ELISA[y]	Healthy[z]
Yolo Wonder	0.645^{ab}	M	0.956^{a}	M	0.800^{a}	0.161
I-20	0.485^{abc}	SMD,M	0.926^{a}	SMD,SM	0.706^{ab}	0.181
PI 159236	0.680^{ab}	MM	0.361^{b}	MM	0.521^{abc}	0.180
Greenleaf Tabasco	0.724^{a}	M	0.131^{b}	MM	0.427^{bcd}	0.164
Casca Dura Ikeda	0.536^{abc}	NS	0.172^{b}	NS	0.354^{cd}	0.143
Magda	0.458^{abc}	NS	0.134^{b}	NS	0.296^{cd}	0.173
Jaloro	0.285^{bc}	NS	0.157^{b}	NS	0.221^{cd}	0.146
PI 152225	0.210^{c}	NS	0.171^{b}	NS	0.190^{d}	0.158
VR4	0.192^{c}	NS	0.178^{b}	NS	0.185^{d}	0.154
VR2	0.176^{c}	NS	0.149^{b}	NS	0.162^{d}	0.191
Delray Bell	0.168^{c}	NS	0.156^{b}	NS	0.162^{d}	0.141
Agronomico 10C-5	0.154^{c}	NS	0.127^{b}	NS	0.141^{d}	0.158

[u]General Linear Models Procedure; Tukey's Studentized Range (HSD) Test was used to compare ELISA absorbance value (405 nm) from different pepper lines and cultivars. Values that share the same letters are not significantly different (p=0.05) from each other.
[v]Combined analysis of ELISA absorbance of experiments 1 and 2.
[w]Mean ELISA absorbance of 4 samples.
[x]Symptom: M=Mosaic, SMD=Severe mosaic and leaf distortion, SM=Severe mosaic, MM=Mild mosaic, NS=No symptoms.
[y]Mean ELISA absorbance of 8 inoculated pepper samples.
[z]Mean ELISA absorbance of 8 healthy pepper samples.

Table 21. Comparison of symptoms and ELISA light absorbance values for leaf extracts from 12 pepper lines inoculated with TEV-H93-5 obtained from two repeated experiments in Phase 3[u]

	Experiment 1		Experiment 2		Experiment 1 & 2[v]	
Pepper lines	ELISA[w]	Symptom[x]	ELISA[w]	Symptom[x]	ELISA[y]	Healthy[z]
I-20	1.692[a]	SMD,SM	0.973[b]	SMD,M	1.332[a]	0.167
Greenleaf Tabasco	1.664[a]	SMD,M	0.771[c]	SMD,S	1.217[ab]	0.161
PI 159236	0.934[c]	SMD	1.232[a]	SMD	1.083[bc]	0.177
PI 152225	1.288[b]	SM	0.563[d]	SM	0.925[c]	0.171
Yolo Wonder	0.689[c]	SMD	0.357[e]	SMD	0.523[d]	0.196
Jaloro	0.291[d]	NS	0.158[f]	NS	0.224[e]	0.165
VR4	0.246[d]	NS	0.179[ef]	NS	0.212[e]	0.184
VR2	0.221[d]	NS	0.167[ef]	NS	0.194[e]	0.208
Magda	0.230[d]	NS	0.148[f]	NS	0.189[e]	0.167
Casca Dura Ikeda	0.212[d]	NS	0.147[f]	NS	0.179[e]	0.152
Delray Bell	0.178[d]	NS	0.139[f]	NS	0.158[e]	0.145
Agronomico 10C-5	0.147[d]	NS	0.117[f]	NS	0.132[e]	0.144

[u]General Linear Models Procedure; Tukey's Studentized Range (HSD) Test was used to compare ELISA absorbance value (405 nm) from different pepper lines and cultivars. Values that share the same letters are not significantly different (p=0.05) from each other.
[v]Combined analysis of ELISA absorbance of experiments 1 and 2.
[w]Mean ELISA absorbance of 4 samples.
[x]Symptom: SMD=Severe mosaic and leaf distortion, SM=Severe mosaic, M=Mosaic, S=Stunt, NS=No symptoms.
[y]Mean ELISA absorbance of 8 inoculated pepper samples.
[z]Mean ELISA absorbance of 8 healthy pepper samples.

Table 22. Results of ELISA tests for samples from 13 pepper lines inoculated with 10 tobacco etch virus isolates in Phase 3 from combined analysis of experiments 1 and 2

	TEV isolates									
Pepper lines	401	DR93 -28	VIL	CAY -90	C1	TX -M	LMS -M	MEX -21	V-92 -4	H-93 -5
Tabasco	NT[a]	NT	NT	NT	NT	NT	NT	NT	NT	NT
Yolo Wonder	+[b]	+	+	+	+	+	+	+	+	+
I-20	+	+	+	+	+	+	+	+	+	+
Greenleaf Tabasco	+	+	+	+	+	+	-[c]	+	+	+
PI 159236	+	+	+	+	+	+	-	+	+	+
VR2	+	+	+	+	+	+	+	+	-	-
Magda	+	+	+	+	-	+	+	+	+	-
Casca Dura Ikeda	+	+	+	+	-	+	+	+	+	-
Jaloro	+	+	+	-	-	+	+	+	+	-
PI 152225	+	+	+	-	+	+	-	+	-	+
VR4	+	+	+	+	+	-	+	-	-	-
Delray Bell	+	+	-	+	+	-	+	-	-	-
Agronomico 10C-5	+	+	-	+	+	-	+	-	-	-

[a]NT = Not tested.

[b]+ = Positive detection of TEV.

[c]- = Negative results (failure to detect TEV).

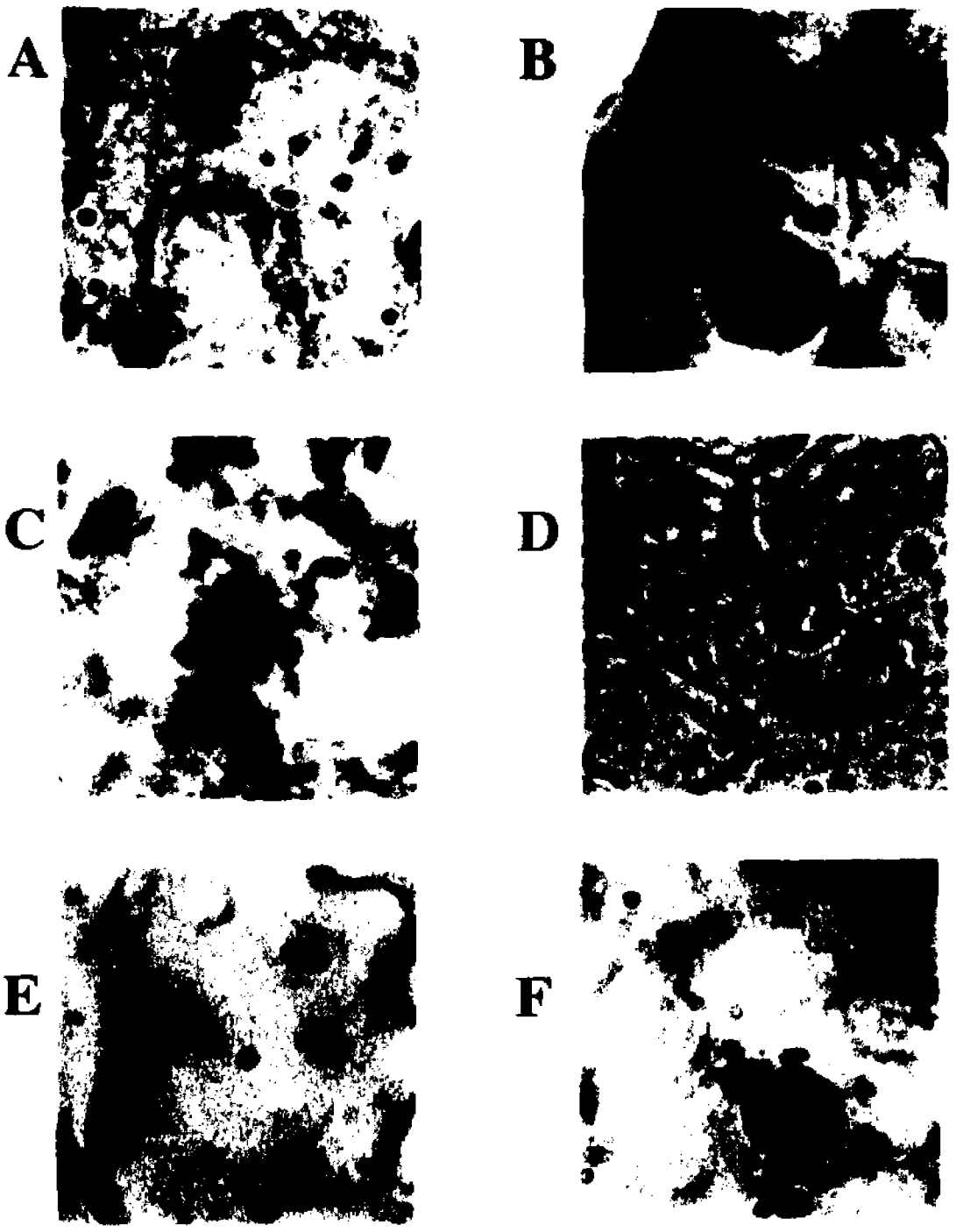


Fig. 6. Nuclear inclusions induced by isolates of TEV in Yolo Wonder pepper. (**A**) Healthy Yolo Wonder cell. (**B**) Square-shaped inclusion induced by TEV-401. (**C**) Folded plate induced by TEV-CAY-90. (**D**) Curved inclusion induced by TEV-MEX-21. (**E**) Needle-like inclusions induced by TEV-LMS-M. (**F**) TEV-H93-5-induced inclusions.

DsRNA analysis. DsRNA from datura infected with all 10 isolates of TEV consisted of a single band with a molecular weight of approximately 6 x 10^6 Daltons (Fig. 7). This dsRNA band was physically identical for all 10 isolates. A dsRNA band of size similar to that of TEV was detected in healthy Yolo Wonder pepper. However, this dsRNA appears to be of non-viral nature (Valverde et al. 1990).

Fig. 7. Polyacrylamide gel (6%) electrophoresis of TEV dsRNA. Lane 1, dsRNA extracted from healthy Yolo Wonder. Lane 2, dsRNA of healthy Yolo Wonder treated with DNase. Lane 3, dsRNA extracted from *Datura stramonium* infected with TEV-H93-5. Lane 4, dsRNA from *D. stramonium* infected with TEV-H93-5, treated with DNase. Lane 5, dsRNA from healthy *D. stramonium* treated with DNase.

DISCUSSION

A number of different TEV strains and isolates have been reported (Smith, 1970; Zitter, 1973; Makkouk and Gumpf, 1974; Villalon, 1985). But prior to the present study there has not been an attempt to differentiate and group a large number of geographically diverse isolates.

The 36 TEV isolates used in this study could be only partially grouped according to symptoms induced on the 13 pepper lines and cultivars in Phase 1. Twenty isolates of TEV formed six groups. Isolates in group 1 (H92-31, 401, C1, CAY-90) were virulent on all the pepper lines tested, and isolates in group 6 (ATCC PV 69 and FL978) exhibited a very limited range of virulence and induced symptoms only in Yolo Wonder, Casca Dura, and Yolo Y. Sixteen of 36 isolates did not fit into any group based on their reaction in the 13 pepper lines. These 16 isolates often differed from members of a group by their ability or inability to infect one line or cultivar.

If isolates from an area are similar it will be easier to develop and successfully employ resistant cultivars. Some isolates collected from the same geographic areas showed similar reactions in Phase 1. Isolates B1 and B3 collected from Louisiana (Group 5), three Dominican Republic isolates (group 3) and two isolates from Florida (Group 6) are some examples. However, other isolates collected from the same geographic area did not induce symptoms in the same pepper lines. Isolate CAJ 2A #1, collected from Louisiana, was included in group 3, whereas isolates B1 and B3 collected from the same area were

included in group 5. Therefore it is possible that different TEV strains may exist in a single pepper field. This adds to the complexity and difficulty of developing resistant cultivars.

Some isolates from different geographic areas were grouped together in Phase 1. Isolate H92-31 from Honduras and isolate 401 from California group 1 behaved similarly. Therefore, it is possible that in some cases common germplasm sources could be utilized for breeding for resistance to TEV isolates from different geographic areas.

Diversity of symptom severity was evident among the TEV isolates used. Examples representing extremes were isolates LMS-M and V92-4. Symptoms induced by LMS-M in most susceptible pepper lines included severe mosaic and leaf distortion. Isolate V92-4 induced mild symptoms on most of the pepper lines it infected.

Pepper lines obtained from the Plant Genetic Resources Conservation Unit showed obvious segregation with respect to resistance/susceptibility reactions to TEV isolates in Phase 2. These lines included some plants that were symptomless while others had moderate or severe symptoms. In contrast, lines derived from seed obtained from other sources showed much more consistent symptomatology.

Results from Phases 1, 2, and 3 were sometimes inconsistent with each other. Pepper line I-20 (AMA 12) showed startling changes in apparent resistance from Phase 2, when it was generally symptomless after inoculation by most isolates, to Phase 3,when it showed symptoms, often severe, after inoculation by most isolates. Other pepper lines and virus isolates showed some inconsistencies between phases, but on a less dramatic level than I-20.

Possible explanations for these inconsistencies include seasonal temperature changes, seasonal light intensity changes, or differences in titer of inoculum. Environmental effects on PVY resistance in pepper were reported by Shifriss and Cohen (1971). Pepper lines resistant to PVY in summer greenhouse tests were susceptible to PVY in an unheated greenhouse and in the field during the winter in Israel. Seasonal changes in temperature and/or light intensity could be involved in I-20's variation in disease reaction. I-20 was generally resistant to most isolates during Phase 2, carried out in July and August of 1994, but was generally susceptible during Phase 3, experiment 1 (November 1994 - January 1995) and Phase 3, experiment 2 (March 1995 - April 1995).

Despite inconsistencies in some virus isolate - pepper line interactions between the different phases, there were fairly consistent trends with respect to virulence of isolates. Consistently among the more virulent isolates were 401, DR93-28, VIL, and TX-M. Virulence in this case is defined as the ability to infect different resistant lines. Among the less virulent isolates was V92-4.

There were also generally consistent trends with respect to resistance of pepper lines through the different phases. Delray Bell, Agronomico 10C-5, VR4, Jaloro, and PI 152225 were among the pepper lines showing symptomless reactions to many of the isolates against which they were tested. These lines would appear to be good TEV resistance sources for pepper breeders looking for resistance useful in broad geographic areas. It should be noted that the Agronomico 10C-5 seed used in Phases 1 and 3 was

originally obtained from PetoSeed Company. Plants from this seed source were consistent in virus reaction, in contrast with the segregating material used in Phase 2 that was obtained from the Plant Genetic Resources Conservation Unit.

Isolates LMS-M and H93-5 gave nearly opposite reactions in the resistant pepper lines of Phase 3. LMS-M induced symptoms, generally severe, in Jaloro, Magda, Casca Dura Ikeda, VR2, VR4, Delray Bell, and Agronomico 10C-5, but not in Greenleaf Tabasco, PI 159236, and PI 152225. Isolate H93-5 induced symptoms in Greenleaf Tabasco, PI 159236, and PI 152225 but not in the other resistant lines mentioned above. This isolate not only shows an interesting contrast with LMS-M, but also contradicts Greenleaf's model of resistance alleles to potyviruses. According to Greenleaf (1986), PI 152225 has a higher level of resistance to TEV than VR2.

ELISA testing of pepper lines inoculated with the various isolates revealed some complex relationships between symptoms and apparent virus concentration, as estimated by ELISA absorbance. As would be expected, plants with symptoms were usually ELISA positive, and most plants without symptoms were ELISA negative. However, in some virus isolate-pepper line combinations, symptomless plants had absorbances more than twice those of healthy control averages, and were therefore ELISA-positive. More surprising, perhaps, were the ELISA-negative plants with symptoms; apparently, despite their symptomatology, the concentration of virus present in the plants was below the threshold of ELISA detection. A similar phenomenon was encountered by Kuhn et al.

(1989) who observed that 18% of plants in a field test of the TEV-resistant line GA-C44-V22 had mild mottle symptoms, but all were ELISA-negative. In addition, 85% of TEV-resistant line FL-XVR-3-25 had mild virus symptoms in the field, but only 8% were ELISA-positive.

Pepper lines GA-C44-V22 and FL-XVR-3-25 were symptomless after mechanical inoculation with TEV in greenhouse tests conducted by Kuhn et al. (1989), but exhibited symptoms after natural infection in the field to the degree cited above. The three more susceptible lines used in their study showed symptoms both in the greenhouse and field tests. Sowell and Demski (1977) tested six TEV resistant or moderately resistant pepper lines in the greenhouse and field. Five of the six gave similar reactions in greenhouse mechanical inoculation and field natural infection tests, but one line was intermediate in greenhouse tests while susceptible in the field. These examples demonstrate that greenhouse testing may not always correlate perfectly with field results for TEV resistance screening in pepper. However, field testing of virus resistance can only be confined to local virus isolates, due to concerns over the effect of releasing non-native isolates on local agriculture.

Because of inconsistencies between results of the three different phases, possibly due to environmental variation, it was not possible to establish clear pathotypes within the virus isolates, nor to choose pepper line differentials that would separate the isolates into pathotypes. However, utilizing 8 of the 13 Phase 3 pepper lines (Yolo Wonder as susceptible control, PI 159236, Jaloro, Magda, PI 152225, VR2, VR4, and Delray Bell) the virus isolates used in Phase 3 can be put in certain categories. Tabasco and I-20 would

not be useful because of susceptibility to all isolates. Casca Dura Ikeda had similar reactions to the isolates as Magda, therefore is not useful. Likewise, Agronomico 10C-5 reacted similarly to Delray Bell so is not needed. Higher virulence isolates would include 401, DR93-28, VIL, TX-M, C1, CAY-90, and MEX-21, as they all induced symptoms on five or more of the eight lines. These seven isolates could be ranked according to the number of lines in which they induced symptoms, although these rankings would change if different resistant lines were used. Isolate V92-4 would be classified as a lower virulence isolate since it induced symptoms on only two of the eight lines. An unusual isolate group would include LMS-M and H93-5. Isolate LMS-M was unable to overcome the *C. chinense* resistance of PI 152225 and PI 159236, but caused symptoms, often severe, on the resistant *C. annuum* lines .Isolate H93-5 was unable to induce symptoms on the resistant *C. annuum* lines, but caused severe symptoms on PI 152225 and PI 159236. Therefore, the eight pepper lines can be used to make general virulence groupings for most isolates, and to define certain unusual isolates like LMS-M and H93-5.

The type of nuclear inclusions could not be used to distinguish most TEV isolates, but the three isolates LMS-M, H93-5, and CAY-90 induced characteristic inclusions. Seven other TEV isolates could be grouped in one category according to the type of inclusions induced.

DsRNA profiles of the same TEV isolates used in the nuclear inclusion studies could not be used to differentiate those isolates. This is not surprising since Valverde et al. (1986) reported that different potyviruses could not be differentiated by dsRNA analysis.

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APPENDIX

Extraction of dsRNA from healthy pepper

The dsRNA profiles of Capsicum accessions with resistance to TEV were evaluated. Thirteen accessions of pepper in three species, *Capsicum annuum*, *C. chinense* and *C. frutescens* used in Phase 3 were selected for pepper dsRNA extraction.

Leaves (3.5 g) from 10 healthy plants of each pepper accession grown in the greenhouse were collected, and dsRNA extraction was performed as described in viral dsRNA extraction. Pellets were resuspended in 200 ul of DNase buffer, added 20 ul of DNase (1mg/1ml), and incubated at room temperature for 15 min. Electrophoresis was conducted as described previously. The gel was stained, observed under UV light transilluminator (300 nm), and photographed using Polaroid film type 57.

Results. Figure A1 shows the polyacrylamide gel after electrophoresis.

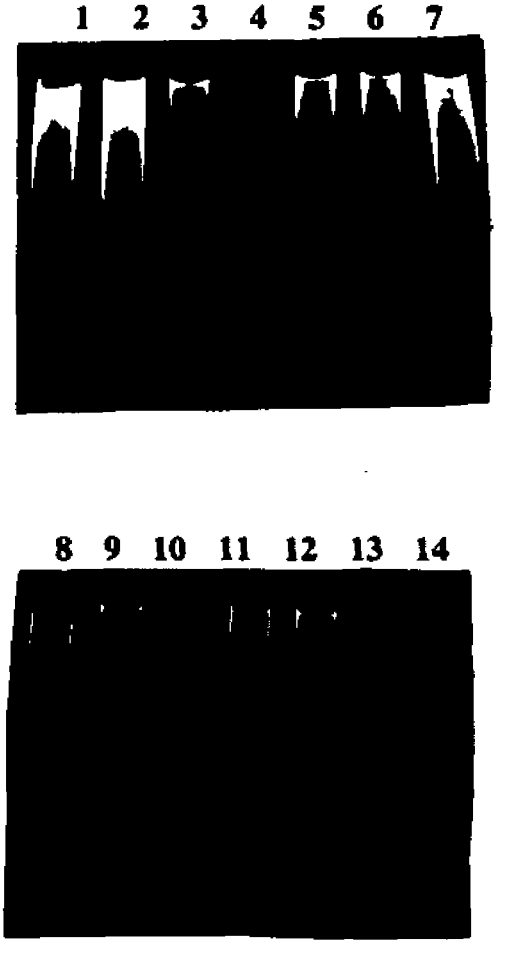
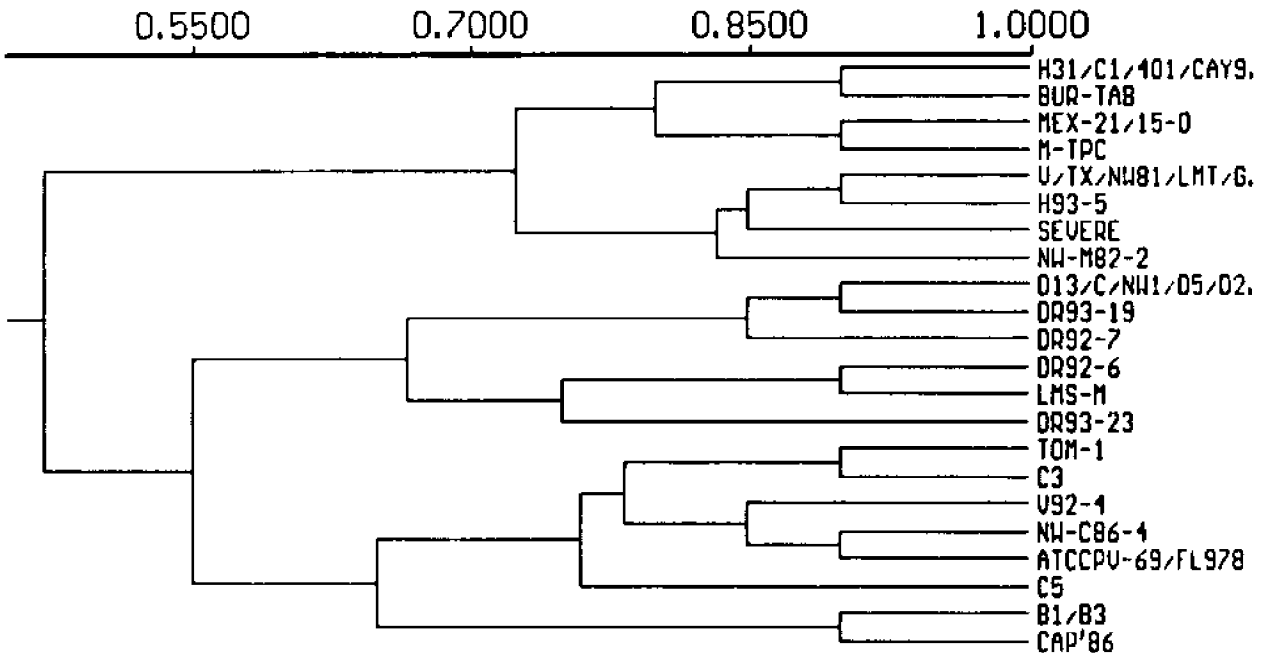


Fig. A 1. Polyacrylamide gel (6%) after electrophoresis of dsRNA from pepper lines and cultivars used for screening isolates of TEV in Phase 3. All the samples were treated with DNase. Lanes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11,12, and 13 show the dsRNA bands (arrow) extracted from Greenleaf Tabasco, Magda, Yolo Wonder, Gallery, PI-159236, VR2, VR4, PI-152225, Delray Bell,I-20, Agronomico-10C-5, Casca Dura Ikeda, and Tabasco respectively. Lane 14 contains dsRNA of tobacco mosaic virus.



H31/C1/401/CAY9 =H-92-31, C1, 401, CAY90
V/TX/NW81/LMT/G =VIL, TX-M, NW-M81, LMTP-M, GLT-F
D13/C/NW1/D5/D2 =DR93-13, CAJ2A#1, NW-M83-1, DR92-5, DR93-28

Fig. A2. A dendrogram of the data in Table 5 showing relationships between the 36 tobacco etch virus isolates used in Phase 1.

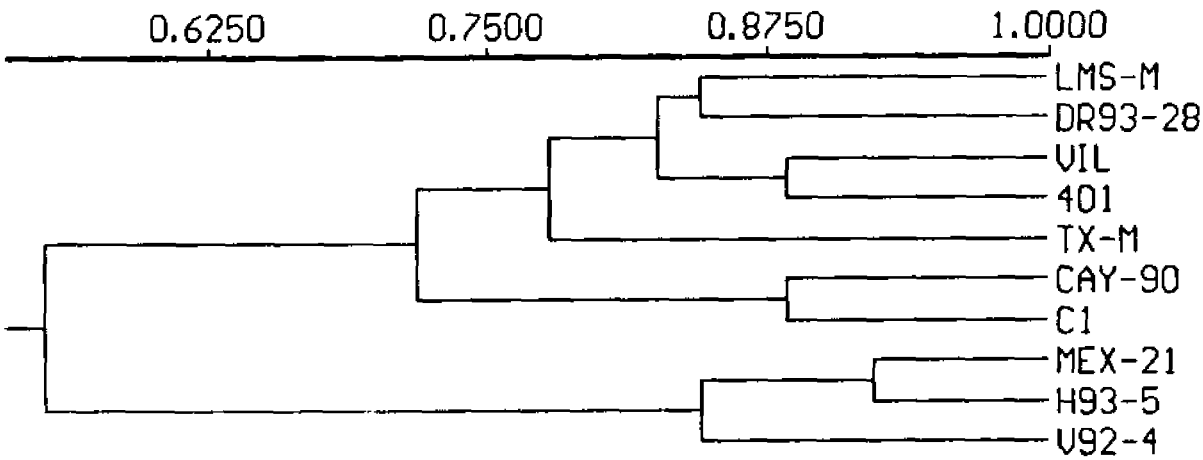


Fig. A3 A dendrogram of the data in Table 8 showing relationships between the 10 tobacco etch virus isolates used in Phase 2.

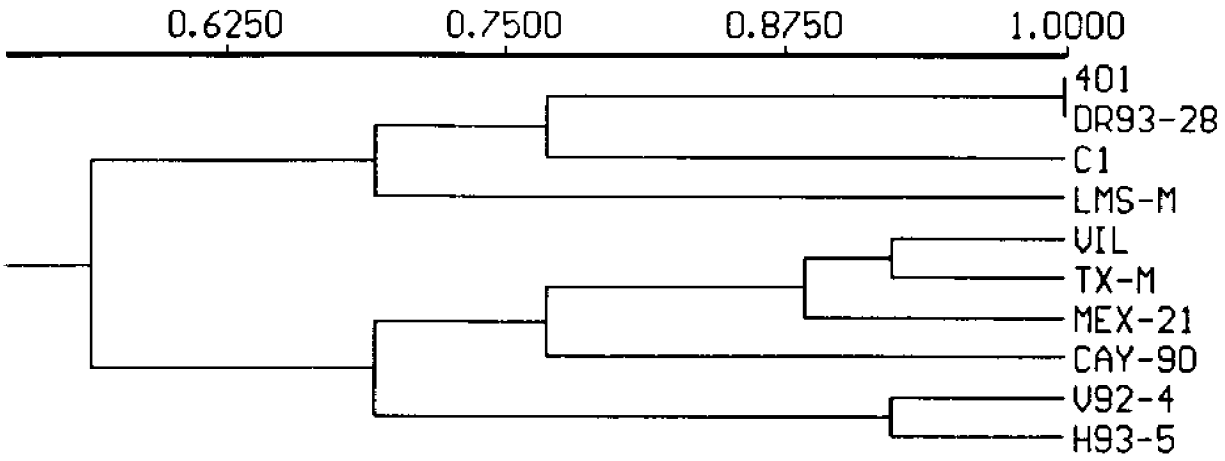


Fig. A4. A dendrogram of the data in Table 10 showing the relationships between the 10 tobacco etch virus isolates used in Phase 3.

VITA

Indra Ariyaratne was born on March 18, 1952, in Aranayake, Sri Lanka. She received her primary and secondary education in Kegalle Convent, Kegalle and Hillwood college, Kandy , Sri Lanka. She continued her studies at the University of Peradeniya from 1975 and received her B.S. degree in Biology in April, 1978.

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